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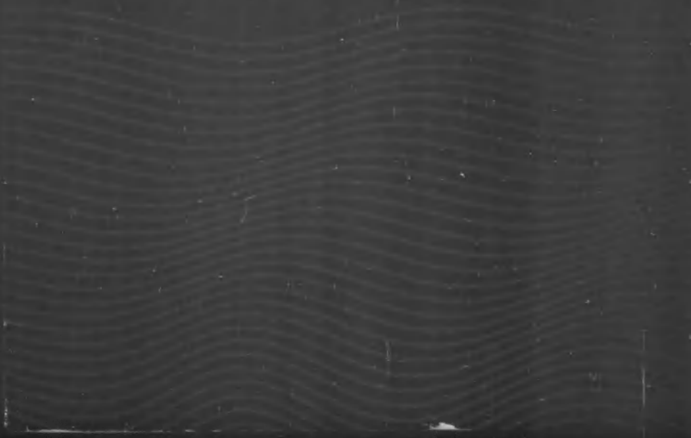

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Estimating Stock Composition in Mixed Stock Fisheries Using Morphometric, Meristic, and Electrophoretic Characteristics

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We describe a conditional maximum likelihood procedure for estimating stock composition in a mixed-stock fishery, provided that samples can be collected from the contributing stocks in isolation from each other and that characters exist that differ significantly between stocks. The procedure presented can use discrete (electrophoretic, meristic) or continuous (morphometric) data or any combination of these data. The procedure is tested by simulations and is used to estimate stock compositions of chum salmon (*Oncorhynchus keta*) sampled in a test fishery near Vancouver Island, B.C., in 1981. The estimated composition in the test fishery agreed closely with the results of previous tagging studies in the same area.

L'article qui suit contient une description d'une méthode de probabilité maximale conditionnelle pour estimer la composition des stocks dans une pêche exploitant des stocks mixtes, à condition qu'on puisse prélever des échantillons des diverses composantes indépendamment les unes des autres et qu'il existe, entre les stocks, des caractères significativement différents. La méthode peut faire appel à des données soit disjointes (électrophorétiques, méristiques), soit continues (morphométriques), ou d'une combinaison quelconque de ces données. Cette méthode a été vérifiée à l'aide de simulations et nous l'avons utilisée pour estimer la composition des stocks de saumons keta (*Oncorhynchus keta*) échantillonnés lors d'une pêche d'essai près de l'Île Vancouver (C.-B.) en 1981. La composition estimée dans cette pêche d'essai s'accorde bien avec les résultats de marquages antérieurs dans la même région.

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One of the most common practices in fishery science is the estimation of a sample's composition by the assignment of the individuals in the sample to particular classes on the basis of a set of biological attributes. Following assignment of all the individuals, the frequency of each class is used to estimate the sample's composition. The assignment of these individuals may be based on implicit predetermined decision rules, as in the visual ageing of scales, or explicit, as with classification functions in stock identification (Mulligan et al. 1983; Cook 1982; Pella and Robertson 1979; Lear and Sandeman 1974; Messinger and Bilton 1974). Estimation of the proportions of the contributing classes in a sample, presumably a "mixture" of classes, via classification is then an indirect, two-step procedure considering each individual in isolation from others in the sample. Alternatively, a direct estimation of the proportions of the contributing classes in a mixed sample (i.e. a mixture) is potentially more powerful than classification approaches because information is gained by considering the mixture as a whole. Alternative approaches that have been suggested include cluster analysis (Wolfe 1970), estimation of the proportions when samples from the contributing classes exist (Milner et al. 1981), and/or imposition of constraints on class limits (Schnute and Fournier 1980).

This paper presents a conditional maximum likelihood method for the direct estimation of the proportions that various populations represent in a fishery, given that samples from *all* contributing populations are available. This problem is referred to as the finite mixture problem with learning samples (FMP-LS). Learning samples are samples drawn from populations of known origin and in isolation from other contributing populations. Our objective with this method was to extend previous work in several ways, notably (i) to be able to use several types of continuous and/or discrete characteristics simultaneously, (ii) to express electrophoretic data as genotypic or allelic frequencies, the latter reducing the number of parameters estimated by the model, (iii) to be able to use morphometric data in determining mixture compositions by incorporating length as a covariate for adjusting morphometric characters to differentiate stocks on the basis of shape, and (iv) to utilize computer simulations to estimate the variances of the estimated proportions.

A central issue in solving any mixture problem is the selection of characters to measure on each individual. The characters must obviously vary significantly among populations relative to their variability within populations, but the desirable attributes of the characters and their selection procedures will not be discussed here. We demonstrate the model by applying it to a mixed-stock salmon fishery off the west coast of British Columbia.

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Methods

Mathematical Formulation of FMP-LS

The fundamental problem addressed is the following: given a collection of fish that contains unknown proportions of fish from various groups (the mixture), together with collections of fish from each of the groups (the learning samples), how can one estimate the proportion of fish from each group that is present in the mixture?

Assume that there are N groups indexed by j and M observed characteristics indexed by i. If a fish is picked at random from the jth group and its characteristics observed, we shall assume that the M-dimensional vector $\boldsymbol{X}$ of observed characteristics is a random vector whose probability density function (hereafter abbreviated to pdf) will be denoted by $f_j(\boldsymbol{X})$.

Let P_j be the proportion of fish in the mixture that comes from the jth group. If a fish is picked at random from the mixture and its characteristics observed, it follows that the M-dimensional vector $\boldsymbol{Z}$ of observed characteristics is a random vector whose pdf is given by

$$\sum_j P_j f_j(\boldsymbol{Z}).$$

Assume that a random sample of fish has been taken from the mixture and the characteristics of the fish observed. Let $\boldsymbol{Z}_r$ denote the vector of observed characteristics from the rth fish. The log-likelihood function associated with the sample is

$$(1.1) \quad \sum_r \log_e \left[\sum_j P_j f_j(\boldsymbol{Z}_r) \right]$$

Estimation of Parameters

If the pdf's $f_j(\boldsymbol{X})$ were known, the maximum likelihood estimates for the proportions P_j would be found by maximizing the log-likelihood function 1.1 with respect to the P_j. In most situations the pdf's are not known and must be estimated. To carry out this estimation procedure it is necessary to make some assumptions about the form of the pdf's. We shall assume that the pdf's f_j can be expressed as functions of the random vector $\boldsymbol{X}$ and a vector of parameters $\boldsymbol{A}_j$. Specifically, we shall assume that

$$(2.1) \quad f_j(\boldsymbol{X}) = g_j(\boldsymbol{X}; \boldsymbol{A}_j)$$

for some unknown value of the parameter vector $\boldsymbol{A}_j$.

It is now necessary to utilize the learning samples to estimate the correct values of the parameter vectors. For each group j assume that a random sample of fish has been taken and the characteristics observed. Let $\boldsymbol{X}_{js}$ denote the vector of characteristics for the sth fish from the jth group. For each group the log-likelihood function for the random samples is given by

$$(2.2) \quad \sum_s \log_e [g_j(\boldsymbol{X}_{js}; \boldsymbol{A}_j)]$$

while the log-likelihood function for the mixture sample is

$$(2.3) \quad \sum_r \log_e \left[\sum_j P_j g_j(\boldsymbol{Z}_r; \boldsymbol{A}_j) \right]$$

The simultaneous likelihood function for all the learning samples and the mixture sample is

$$(2.4) \quad \sum_j \sum_s \log_e [g_j(\boldsymbol{X}_{js}; \boldsymbol{A}_j)] + \sum_r \log_e \left[\sum_j P_j g_j(\boldsymbol{Z}_r; \boldsymbol{A}_j) \right].$$

The maximum likelihood estimates for the parameters are found by maximizing expression 2.4 with respect to the $\boldsymbol{A}_j$ and $\boldsymbol{P}_j$.

In this paper we have not computed the maximum likelihood estimates. Instead we have chosen the computationally simpler approach of using conditional maximum likelihood estimates. These are calculated by first maximizing expression 2.2 with respect to the $\boldsymbol{A}_j$ for each group. These parameter estimates denoted by $\hat{\boldsymbol{A}}_j$ are then substituted into expression 2.3 yielding expression 2.5:

$$(2.5) \quad \sum_r \log_e \left[\sum_j P_j g_j(\boldsymbol{Z}_r; \hat{\boldsymbol{A}}_j) \right].$$

The conditional maximum likelihood estimates $\hat{P}_j$ for the mixture problem are found by maximizing expression 2.5 with respect to the P_j subject to the constraints $P_j \geqslant 0$ and

$$\sum_j P_j = 1.$$

The conditional maximum likelihood estimates are inferior to maximum likelihood estimates in that they ignore the extra information about the parameter vectors $\boldsymbol{A}_j$ that is present in the mixture sample.

The statement of the problem as given is very general. The real structure is introduced by making assumptions about the exact form of the pdf's $g_i(\boldsymbol{X}; \boldsymbol{A}_j)$.

Derivation of the pdf's

Assumption of independence

For each group we shall assume that the components of the random vector are pairwise independent random variables. From this assumption it follows that the pdf $g_j(\boldsymbol{X}; \boldsymbol{A}_j)$ can be expressed as a product:

$$(3.1) \quad g_j(\boldsymbol{X}; \boldsymbol{A}_j) = \prod_i h_{ij}(x_j; \boldsymbol{B}_{ij})$$

where h_{ij} is the pdf for the ith characteristic from the jth group.

In expression 3.1 the $\boldsymbol{B}_{ij}$ denote the subset of the parameter vector $\boldsymbol{A}_j$ associated with the pdf for each characteristic. Note that different characteristics do not share common parameters. This assumption is not crucial to the analysis, but it simplifies the parameter estimation procedure and is usually satisfied in practice.

Not all characteristics will be independent, however, but independence between characteristics selected for inclusion in the analyses was assumed in order to minimize the number of parameters in the model while still adequately describing the phenomena being investigated. Consider two discrete characteristics that can take on k and l distinct states, respectively. If characteristics are independent the pdf for the random vector consisting of the pair of observations can be specified by $k + l - 2$ parameters. If characteristics are not independent, then since there are kl possible pairs of observations, it will require $kl - 1$ parameters to specify the pdf for the random vector of pairs of observations. With more than two characteristics the number of parameters grows exponentially with the number of characteristics. With such a large number of parameters the model can lose its ability to identify all the parameter values.

When characteristics are only moderately correlated, the assumption of independence should not cause much loss of efficiency in the estimation procedure. Very highly correlated characteristics can be grouped so that each set of observations of them is treated as a single characteristic in the model.

Discrete and continuous random variables

The form of the pdf for a characteristic depends on whether it is a discrete or continuous characteristic. A discrete character is distributed acorss the $x_1, \ldots, x_n$ possible states according to its probability mass function (pmf). Let the pmf $h(x_i; \mathbf{B})$ be the probability that the random variable X takes on state x_i. Thus, $h(x_j; \mathbf{B})$ is determined by the $n - 1$ parameters $B_1, \ldots, B_{n-1}$ where $h(x_i; \mathbf{B}) = B_i$ for $i = 1, \ldots, n - 1$ and

$$h(x_n; \mathbf{B}) = 1 - \sum_{i=1}^{n-1} B_i.$$

It may be possible for certain characteristics to introduce extra structure into the model that will reduce the number of parameters estimated. This is done by reparameterizing the B_i such that they depend on a smaller number of parameters. For example, when parameterizing the pmf for discrete electrophoretic characteristics this reduction of the number of parameters is based on Mendelian inheritance. If the k states of an electrophoretic characteristic are in fact genotypes, then each state is determined by a pair of alleles. Assuming that the breeding population from which the fish originated is in Hardy–Weinberg equilibrium (Hartl 1980), then the pmf for the electrophoretic character is determined by the pmf for the allele type as follows. If there are p alleles determining the genotypic states of an electrophoretic characteristic, there are $p(p + 1)/2$ distinct allele pairs (order of the pairs is assumed to be irrelevant). The number of parameters corresponding to that characteristic will, therefore, be reduced from $(p(p + 1)/2 - 1)$ to $p - 1$. If $C_1, \ldots, C_p$ are the probabilities of occurrence of the allele types (i.e. frequency of alleles), then the pmf for an electrophoretic character is given by

$$(4.1) \quad \begin{array}{ll} B_s = C_i^2 & \text{if } i = j \\ \text{or} \quad B_s = 2C_iC_j & \text{if } i \neq j \end{array}$$

where i and j are the indices of the two alleles that determine the sth genotype.

For a continuous characteristic the pdf $h(x; \mathbf{B})$ is determined by the property that the integral

$$\int_{x_1}^{x_2} h(x; \mathbf{B})dx$$

is the probability that the random variable x takes on a value lying between x_1 and x_2.

There are of course many functional forms that can be chosen for the pdf of a continuous random variable. In the present case all continuous characteristics are morphometric characteristics used to characterize fish in groups based on differences in body shape. To compare the shape of fish it is necessary to account for variability in body size of fish sampled in different populations. A common method of doing this is to standardize length of fish within and between groups (Thorpe 1976; Gould 1966), but standardization is not necessary in this model.

Let x denote some morphometric characteristic. For each group j we shall assume that $\log (x)$ is normally distributed with mean $\log (a_j) + b_j \log (L)$ and variance σ_j^2, where L is the length of the fish for which the morphometric characters were observed. The pdf is given by

$$\begin{aligned} h_j(\log (x); \log a_j, b_j, \sigma_j, L) &= 1/(\sqrt{2\pi}\sigma_j) \\ &\times \exp (-(\log (x) - \log a_j - b_j \log (L))^2/2\sigma_j^2). \end{aligned}$$

Note that in this case the pdf depends on three unknown parameters, $\log a_j$, b_j, and σ_j.

Using learning samples to estimate parameters

The method for using the learning samples to estimate parameter values depends on the form of the characteristic. For a discrete characteristic such as a meristic trait, $\hat{B}_{js}$ is the proportion of the sample in which the character takes on the sth state in the jth learning sample group. Then $\hat{B}_{js}$ is the maximum likelihood estimate for $\hat{B}_{js}$ when only the learning sample data are used. If the discrete characteristic considered is the genetic variability of an enzyme and a genetic model for the inheritance of the enzyme has been established, $\hat{C}_{jp}$ is the proportion of the sample in which the pth allele occurs for the jth learning sample group. If the assumption of Hardy–Weinberg equilibrium holds, then the maximum likelihood estimate for the B's can be calculated by expression 4.1. Note that if for a specific enzyme the genetic model is unknown and/or the Hardy–Weinberg condition does not hold, then frequencies of the states observed on the electrograms can be used without estimation of allele frequencies. $\hat{C}_{jp}$ would then be analogous to the $\hat{B}_{js}$ above.

For morphometric characteristics the maximum likelihood estimates for the parameters (a_j, b_j, σ_j^2) are found by linear regression of the logarithm of the morphometric character on the logarithm of the length of the fish from which the character was measured. Many morphological traits follow allometric growth curves with respect to length; consequently, log–log regressions would likely provide the best estimates of a_j, b_j, σ_j^2. However, if a nonlinear regression provided a better fit to the data for a certain trait, then alternative parameters could be estimated, provided that the same set of parameters are applied to all learning sample groups for that trait.

In applying this model it is essential to test the assumptions. These are chi-square tests for the goodness-of-fit of electrophoretic data to Hardy–Weinberg equilibrium and the use of residual and normality plots to assess whether the morphometric traits are distributed normally. A learning sample group that does not meet these assumptions may result in biased estimates of the contribution from this group; however, the degree of bias would have to be assessed within the particular application of this procedure.

Estimation of the P_j's

Assume that the parameters determining the pdf's of the characteristics have been estimated from the learning samples. To estimate the P_j it is necessary to maximize expression 2.5 with respect to the P_j subject to the constraints

$$(5.1) \quad \sum_j P_j = 1 \quad \text{and} \quad P_j \geqslant 0.$$

This is a constrained nonlinear optimization problem. To solve this problem we have employed a nonlinear optimization routine, FMIN, supplied by the computing center of the University of British Columbia. FMIN is a quasi-Newton method that requires the user to supply values of the function to be maximized together with its first derivatives. FMIN is designed to solve unconstrained optimization problems. To eliminate the constraints 5.1, it is necessary to reparameterize the P_j's.

For each j let

$$P_j = \exp (u_j)/\sum_k \exp (u_k).$$

In terms of the u_j, expression 2.4 becomes

$$(5.2)\quad \sum_r \log_e \left[\sum_j \exp(u_j) f_j(Z_r)\right] - \sum_r \log_e \left(\sum_k \exp(u_k)\right).$$

The constraints 5.1 are now automatically satisfied and can be ignored. To solve the problem we could maximize expression 5.2 but there is one final difficulty. If each u_j is replaced by $u_j + c$ for any constant c, the expression 5.2 is left invariant. This degeneracy can lead to problems with the function maximizing routine. To remove this degeneracy an extra expression is added to expression 5.2 leading to expression 5.3:

$$(5.3)\quad \sum_r \log_e \left[\sum_j \exp(u_j) g_j(Z_r; \hat{A}_j)\right] - \sum_r \log_e \left(\sum_j \exp(u_j)\right) - \left[\log_e \left(\sum_j \exp(u_j)\right)\right]^2.$$

The u_j that maximize 5.3 correspond to those particular u_j that maximize expression 5.2 and also satisfy the condition

$$\sum_j \exp(u_j) = 1.$$

It is also necessary to provide an initial value of the u_j. As initial values for the u_j we set $u_j = \log(1/N)$.

Estimating the Covariance Matrix of the Parameter Estimates

The general reference for this procedure is Bard (1974). To estimate the variance of the parameter estimates we have employed the method of computer simulation (Bard 1974, p. 46–47). We have eschewed the use of the approximate analytic techniques involving the second derivatives of the log-likelihood function for the following reasons.

Because of the nature of the problem, the conditional maximum likelihood solution often lies close to the constraints $P \geqslant 0$. As Bard (1974, p. 181) pointed out, this makes it difficult to compute the covariance matrix of the parameter estimates by approximate analytic techniques. Also, since we have used the conditional maximum likelihood estimates, the approximate analytic techniques give variance estimates for the $\hat{P}_j$ under the assumption that the parameters A_j determining the characteristics are known without error. As the simulations show, a large part of the variance of the $\hat{P}_j$ is caused by uncertainty in the estimates of the A_j, so that use of the approximate analytic techniques would tend to give unreasonably optimistic estimates of the covariance matrix.

Uniqueness of the Conditional Likelihood Estimates

It is well known that the likelihood function for finite mixture problems may have numerous local maxima. Although nothing can be said in general about the uniqueness of the conditional likelihood estimates, it can be shown that for "reasonable" problems, the conditional maximum likelihood estimates are unique. The general reference for this is Panik (1976).

Proposition 1

The set of points $(P_1, \ldots, P_N)$ satisfying the conditions

$$\sum_j P_j = 1$$

and $P_j \geqslant 0$ form a closed bounded convex subset of ξ^N. The proof is simple and hence will be omitted.

Theorem 8.3 (Panik)

A real valued function is convex if the Hessian (matrix of second partial derivatives) is negative semidefinite. If the Hessian is negative definite the function is strictly convex.

Theorem 8.4 (Panik)

Let a real valued function f be strictly convex over a closed convex set in $\mathscr{E}^N$. Then f assumes a strong global maximum somewhere on its domain. This extremum is unique.

For each r (recall that r indexes the fish in the mixture sample), let $G_r = (g_1(\mathbf{Z}_r; \mathbf{A}_1), \ldots, g_n(\mathbf{Z}_r; \mathbf{A}_N))$.

Proposition 2

In order that the Hessian of expression 2.5 be negative definite (when expression 2.5 is regarded as a function of the P_j) it is sufficient that the vectors $\mathbf{G}_r$ span the N-dimensional space $\mathscr{E}^N$. *Proof*: Let $\mathbf{X} \cdot \mathbf{Y}$ denote the scalar product of vectors $\mathbf{X}$ and $\mathbf{Y}$ in $\mathscr{E}^N$ (Panik 1976, p. 10). Let $\mathbf{H}(\mathbf{P})$ denote the Hessian matrix of expression 2.5 at the point $P = (P_1, \ldots, P_N)$. By definition (Panik, p. 42), $\mathbf{H}(\mathbf{P})$ is negative definite if $\mathbf{h}'\mathbf{H}(\mathbf{P})\mathbf{h} < 0$ for all nonzero vectors $\mathbf{h}$ in $\mathscr{E}^N$. Now,

$$\mathbf{h}'\mathbf{H}(\mathbf{P})\mathbf{h} = -\sum_r (\mathbf{G}_r \cdot \mathbf{h})^2 / (\mathbf{P} \cdot \mathbf{h})^2.$$

Thus, $\mathbf{h}'\mathbf{H}(\mathbf{P})\mathbf{h} < 0$ and $\mathbf{h}'\mathbf{H}(\mathbf{P})\mathbf{h} = 0$ if and only if $\mathbf{G}_r \cdot \mathbf{h} = 0$ for all r. This will imply that $\mathbf{h} = 0$ exactly when the vectors $\mathbf{G}_r$ span $\mathscr{E}^N$.

It follows that if the $\mathbf{G}_r$ satisfy the spanning condition then the conditional maximum likelihood estimates will be unique provided that the $\hat{\mathbf{A}}_j$ are uniquely determined. This will of course depend on the particular form of the pdf's, but for the characteristics used in this paper, the $\hat{\mathbf{A}}_j$ will usually be uniquely determined.

Testing of the Model

Actual and simulated fishery data were used to evaluate the model, since different inferences about the expected performance of the model can be drawn. One can determine whether the results from an actual fishery analysis appear reasonable, but since the true composition of the sample is unknown, it is impossible to evaluate the actual performance of the model. The performance of the model with simulated data can be evaluated, but this does not ensure that similar performance will occur with different data. If the model performs well for simulated data and gives reasonable and consistent results for actual data, then it may be expected to yield useful results during application. Finally, by performing a number of simulations, one can estimate the standard deviations of the parameter estimates.

Description of the data

In the autumn of 1981, adult chum salmon (*Oncorhynchus keta*) were sampled from 12 spawning populations (i.e. the learning sample groups) in three regional groupings): 625 from five tributaries of the Fraser River (Harrison, Inch, Squakum, Vedder, and Weaver), 753 from five Vancouver Island rivers (Chemainus, Nanaimo, Little Qualicum, Big Qualicum, and Puntledge), and 200 from the Cheakamus and Sliammon rivers on the east side of the Strait of Georgia (Fig. 1). In addition to the spawning ground sampling, 576 chum salmon were sampled from a test fishery conducted within a 15-km radius from the mouth of the Big Qualicum River during a 4-wk period from October 21 to November 13, 1981.

Characters that measured all the learning samples and that were used in the mixture analyses included postorbital–hypural length, postorbital head length (Vladykov 1962), length of dorsal fin base, the number of gill rakers on the most anterior left gill arch, and the genotypic frequencies for nine enzyme loci. Detailed morphological data from the populations surveyed are outlined in Beacham et al. (1983a) and electrophoretic data are

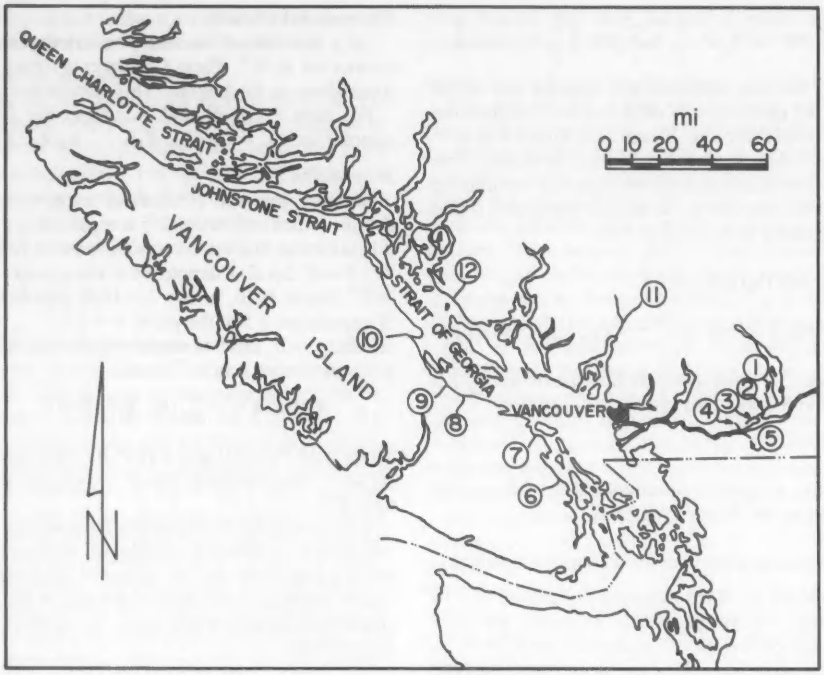

FIG. 1. Location of sites sampled for chum salmon (*Oncorhynchus keta*) in 1981. Areas sampled were (1) Weaver Cr., (2) Harrison R., (3) Squakum Cr., (4) Inch Cr., (5) Vedder R., (6) Chemainus R., (7) Nanaimo R., (8) Little Qualicum R., (9) Big Qualicum R., (10) Puntledge R., (11) Cheakamus R., and (12) Sliammon R. (1 mi = 1.609 km).

outlined in Beacham et al. (1983b). Sexual dimorphism of morphometric traits was observed in these populations. Consequently, when morphometric data were used in the mixture analyses, mixtures of male and female fish were analyzed separately.

Log–log regressions of dorsal fin length and postorbital head length versus body length were significant in all the learning samples and no patterns in the residuals were observed. Electrophoretic data fit Hardy–Weinberg expectations very closely with only 2 of 75 comparisons deviating significantly from expected values. These deviations were observed in two isocitrate dehydrogenase (EC 1.1.1.42) loci in two different populations. The magnitude of the deviations were small and subsequently were ignored when comparisons were made of the model's performance with and without estimating allele frequencies. If all nine loci analyzed had been polymorphic in all 12 populations, a total of 108 tests of the hypothesis of Hardy–Weinberg equilibrium could have been conducted. The difference between tests conducted and those possible is due to monomorphic loci in some populations.

Simulations

Simulated mixed-fishery samples of 500 fish were generated from a hypothetical mixture of 30% Little Qualicum, 60% Big Qualicum, and 10% Puntledge fish using the electrophoretic phenotypic frequencies observed in the spawning ground samples. The compositions of these mixtures were estimated by two different methods.

In the first type of analysis, simulated learning samples of 200 fish from each of the 12 sampled populations were generated, using the observed spawning ground phenotypic frequencies. The entire procedure, including generation of learning samples, generation of the mixture, and analysis of the mixture, was repeated 100 times. In the second type of analysis, learning samples were not simulated. The phenotypic frequencies actually observed in the 12 learning sample groups were used to estimate the mixture compositions. Simulation and analysis of the mixture were repeated 100 times.

The first type of analysis was intended to mimic the actual use of the model in a fisheries analysis (i.e. finite sampling of learning samples), whereas the second analysis might reflect results obtainable if "true" values of the alleic frequencies were known. A comparison of the results from these two analyses indicates the maximum advantage to be gained by increasing the precision of the estimates of allele frequencies in the learning samples and, further, illustrates the bias due to finite learning sample size.

Results and Discussion

Simulation

Direct estimation of the mixture composition determined that 90% of the simulated mixed sample originated from Vancouver Island populations (Table 1). The actual composition was 100% Vancouver Island populations, but given the limited electrophoretic variation between all 12 populations sampled (Table 2), the accuracy of the 90% estimate is considered to be acceptable. The model was not able to estimate the proportional contribution of populations within regional groupings as accu-

TABLE 1. Mean (±1 SE) stock compositions (over 100 stimulations) from the simulated mixture composed of 60% Big Qualicum River, 30% Little Qualicum River, and 10% Puntledge River fish.

Group	True composition	Composition using estimated learning samples		Composition assuming known characteristics in learning samples	
		Mean	SE	Mean	SE
Fraser River group	0.0	0.066	0.006	0.019	0.003
Weaver		0.037	0.0054	0.011	0.0022
Harrison		0.017	0.0029	0.006	0.0018
Squakum		0.001	0.0004	0.000	0.0001
Inch		0.000	0.0002	0.000	0.0000
Vedder		0.009	0.0002	0.002	0.0005
Vancouver Island	1.0	0.900	0.007	0.967	0.004
Chemainus	0.0	0.014	0.0029	0.006	0.0014
Nanaimo	0.0	0.013	0.0026	0.002	0.0008
Little Qualicum	0.3	0.260	0.0089	0.301	0.0083
Big Qualicum	0.6	0.495	0.0099	0.575	0.0078
Puntledge	0.1	0.115	0.0076	0.082	0.0062
Eastern Straits	0.0	0.039	0.004	0.013	0.002
Cheakamus		0.007	0.0014	0.003	0.0011
Sliammon		0.032	0.0042	0.010	0.0002

rately as the between region contribution. This would be expected if the similarity of populations within groups exceeded the similarity of populations between groups. Average pairwise distance within and between regions for electrophoretic data (Table 2) indicates that within-region similarity is approximately twice that of the between-region comparisons. Estimates of the contributions by populations were properly ranked and were within about 1.0 SE of the actual value (Big Qualicum River upper range was 0.594 when the actual value was 0.60). Estimates of the contributions by Big Qualicum, Little Qualicum, and Puntledge rivers were all within ±15–20% of the actual value.

If the learning sample frequencies are known without error, the accuracy and precision of the estimated contribution of each region to the mixture are significantly improved (Table 1). The accuracy of the estimated contributions by populations was similarly improved but the precision on the estimate was not improved equally in all populations. The limited reduction in precision from simulated learning samples to assuming known characteristics in learning samples is probably due to the simulated samples already being large for electrophoretic data. Estimating the probabilities of occurrence of characteristics in learning samples with minimal standard errors would be expected to improve the capability of the model to estimate the contributions of populations, since parameters selected for inclusion in the model should vary significantly between populations relative to variability within the populations. Large learning samples are, therefore, desirable for the selection of variables most useful in separating populations. How large a learning sample should be, however, will depend on how different the populations in question are relative to the variability of the character used and the level of precision desired in the mixture analyses. The researcher should also be aware of alternative strategies for achieving the accuracy and precision desired in the mixture analyses. For example, if the logistics of large samples for a particular character are impractical, sampling alternative characters independent of the previous character(s) may be a more efficient sampling procedure. Sample sizes may also be increased by accumulating smaller annual samples if annual variability is not significant. Table 1 also indicates that bias will likely be introduced to the analyses presumably due to finite learning samples and the use of conditional likelihood analyses. In any application of the model, we recommend the use of simulations, using the observed samples sizes and estimated pdf's as if they were "true" population structures, to assess bias and in determining expected levels of precision in the mixture analyses. Efron (1982) provided a useful description of resampling procedures in the examination of bias and the estimation of sampling variances.

Analysis of Mixed-Stock Fishery Example

East coast Vancouver Island chum populations were estimated to contribute 83–85% (average values within data types 4 and 5 in Table 3 and 4) of the catch in the Big Qualicum test fishery. This contribution appears to be reasonable given the proximity of the test fishery to these stocks and is consistent with previous tagging results. Anderson and Beacham (1983) reported that at least 95% of the chum salmon tagged in the Big Qualicum area were from mid-Vancouver Island rivers. The differences in these estimates may be real but the potential for biased recoveries of tags in rivers proximal to the tagging area should also be noted.

Estimates of the contribution of each population to the test fishery are quite variable depending on sex and data type used in the analyses. Different population contributions by sex may be realistic, as male salmon are known to precede females into spawning streams. Therefore, differential contributions of sexes within a population may reflect differences in timing of spawning migrations through the fishery site. The inconsistency of the estimated contributions within sexes and between data tyupes is not easily explained. However, most inconsistencies occur between populations within a region and are probably due to the inability of the model to differentiate these populations. In

TABLE 2. Genetic and morphological distance measures between 12 populations of chum salmon. Above the diagonal is Nei's (1978) unbiased genetic distance and below the diagonal is the Mahalanobis "D" measure for morphological data (Beacham et al. 1983a, 1983b).

Grouping		Population 1	2	3	4	5	6	7	8	9	10	11	12
East Vancouver Island	1 L. Qualicum	—	0.000	0.000	0.001	0.002	0.001	0.001	0.002	0.002	0.003	0.000	0.001
	2 B. Qualicum	0.86	—	0.000	0.000	0.001	0.000	0.002	0.001	0.001	0.001	0.000	0.001
	3 Puntledge	0.96	0.99	—	0.000	0.001	0.000	0.000	0.001	0.001	0.002	0.000	0.001
	4 Nanaimo	1.95	1.59	1.60	—	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.001
	5 Chemainus	1.56	1.60	1.86	2.12	—	0.001	0.001	0.001	0.002	0.002	0.001	0.003
Fraser River	6 Weaver	1.11	1.45	1.46	1.77	1.26	—	0.001	0.000	0.000	0.000	0.000	0.001
	7 Vedder	1.30	1.55	1.21	1.77	1.46	0.90	—	0.001	0.000	0.003	0.001	0.003
	8 Harrison	1.21	1.54	1.65	2.03	1.05	0.80	0.90	—	0.000	0.000	0.001	0.001
	9 Squakum	1.33	1.74	1.70	2.03	1.28	1.30	1.22	0.94	—	0.000	0.001	0.000
	10 Inch	1.36	1.45	1.79	2.22	1.07	1.21	1.25	0.65	1.33	—	0.002	0.001
Eastern Strait	11 Sliammon	1.31	1.56	1.98	2.53	1.47	1.61	1.83	1.17	1.46	1.21	—	0.001
	12 Cheakamus	1.10	1.35	1.72	2.09	1.66	1.22	1.40	0.99	1.25	1.17	1.05	—

TABLE 3. Estimated proportional contributions of 12 populations of female chum salmon to the catch of female chum salmon ($N = 288$) in the Big Qualicum test fishery.

		Data types for analysis				
Group	Population	Electrophoretic[a]	Morphometric[b]	Electrophoretic[c]	Combined[d]	Combined[e]
Fraser River	Weaver	0.000	0.000	0.000	0.000	0.000
	Harrison	0.001	0.000	0.025	0.001	0.022
	Squakum	0.000	0.001	0.000	0.000	0.001
	Inch	0.001	0.026	0.001	0.001	0.000
	Vedder	0.055	0.000	0.001	0.014	0.002
Group total		0.057	0.027	0.027	0.016	0.025
Vancouver Island	Chemainus	0.032	0.000	0.000	0.016	0.003
	Nanaimo	0.000	0.006	0.000	0.029	0.001
	L. Qualicum	0.006	0.683	0.080	0.090	0.280
	B. Qualicum	0.000	0.019	0.001	0.015	0.018
	Puntledge	0.690	0.002	0.777	0.587	0.459
Group total		0.728	0.710	0.858	0.737	0.761
Eastern Strait	Cheakamus	0.203	0.262	0.113	0.243	0.209
	Sliammon	0.010	0.001	0.001	0.004	0.005
Group total		0.213	0.263	0.114	0.247	0.214

[a] Electrophoretic data, no genetic hypothesis.
[b] Morphometric and meristic data.
[c] Electrophoretic data with genetic hypothesis.
[d] All data, no genetic hypothesis.
[e] All data with genetic hypothesis.

such situations it will likely be necessary to pool populations and discuss a regional contribution to a fishery, as we did earlier.

In both the male and female analyses when electrophoretic data only was considered, estimated proportions of Vancouver Island chum salmon in the test fishery were 17% greater under the hypothesis that the populations sampled were in Hardy–Weinberg equilibrium than when electrophoretic phenotypes were used simply as character states. Electrophoretic data analyzed as allele frequencies (i.e. data type 3) are more consistent with the estimated contributions derived from the morphometric and meristic data (81.4 vs. 84.9% male plus female contributions from Vancouver Island group, respectively) than electrophoretic data analyzed as phenotypic character states (69.05%). The reduction in the number of parameters from the number of phenotypic states to the number of alleles appears to have improved the accuracy of the estimated stock contributions in this example.

The model appears to have worked well in this particular example and the analytic procedure can be readily adapted to other data sets with the same and/or different variables. The success of the method depends largely, however, on the nature, quality, and reliability of the data. The method is likely to give imprecise or biased results if learning sample sizes are small, repeatability of the pdf within populations over time is poor, model assumptions are not valid, variation among learning sample pdf's are limited, or not all groups present in the mixture are represented in learning samples. Individuals using this

TABLE 4. Estimated proportional contribution of 12 populations of male chum salmon to the catch of male chum salmon ($N = 288$) in the Big Qualicum test fishery.

		Data types for analysis				
Group	Population	Electrophoretic[a]	Morphometric[b]	Electrophoretic[c]	Combined[d]	Combined[e]
Fraser River	Weaver	0.001	0.000	0.001	0.000	0.000
	Harrison	0.000	0.008	0.000	0.054	0.056
	Squakum	0.000	0.000	0.006	0.000	0.000
	Inch	0.000	0.001	0.000	0.000	0.000
	Vedder	0.079	0.001	0.029	0.008	0.001
Group total		0.080	0.010	0.036	0.062	0.057
Vancouver Island	Chemainus	0.026	0.000	0.000	0.000	0.000
	Nanaimo	0.037	0.000	0.030	0.011	0.000
	L. Qualicum	0.001	0.618	0.071	0.261	0.432
	B. Qualicum	0.000	0.013	0.000	0.094	0.042
	Puntledge	0.589	0.357	0.669	0.564	0.466
Group total		0.653	0.988	0.770	0.930	0.940
Eastern Strait	Cheakamus	0.264	0.001	0.192	0.005	0.001
	Sliammon	0.001	0.001	0.001	0.001	0.001
Group total		0.265	0.002	0.193	0.006	0.002

[a] Electrophoretic data, no genetic hypothesis.
[b] Morphometric and meristic data.
[c] Electrophoretic data with genetic hypothesis.
[d] All data, no genetic hypothesis.
[e] All data with genetic hypothesis.

TABLE 5. Percent group composition of 288 male chum salmon sampled in the Big Qualicum test fishery as determined with morphometric and meristic data.

Area	Direct estimation	Geisser	Mahalanobis (BMDP7M)
Vancouver Island	99	57	53
Fraser River	1	30	34
Mainland	0	13	13

procedure must be responsible for addressing these sampling concerns, since their impact will vary with particular applications.

Comparison of Classification versus Direct Mixture Method

Two classification methods were evaluated to compare with the direct estimation of stock portions estimated using morphometric and meristic data. Geisser (1964) classification, as outlined by Cooley and Lohnes (1971), and classification based on minimum Mahalanobis distance (BPDP7M) (Dixon et al. 1981) were used. The proportions of each group in the big Qualicum test fishery sample of males were subsequently estimated by summing individuals classified to each group. No allometric corrections for variation in body size were undertaken, since test fishery samples of unknown stock origins cannot be assigned a known population allometric correction.

Estimated stock compositions based on the model we have outlined here indicated that 99% of the males were derived from Vancouver Island rivers, but both classification methods indicated that less than 60% were so classified (Table 5). There was close agreement between estimated stock compositions based on the two classification methods, but they were not in agreement with stock compositions derived from direct estimation.

Estimated stock composition of the test fishery, based solely on electrophoretic character, was obtained from the model and from discriminant analysis. The electrophoretic data was transformed as outlined by Smouse et al. (1982) for the discriminant analysis. About 90% of the chum salmon were classified as originating from Vancouver Island with discriminant analysis based on the electrophoretic data. Direct estimation of mixture components using electrophoretic data and assuming that the breeding populations were in Hardy–Weinberg equilibrium attributed 81.4% of the test fishery to the Vancouver Island group.

The populations we sampled appear to be sufficiently distinct so that classification using categorical data provides similar stock composition estimates as derived from our proposed procedure. Electrophoretic data may be well suited to classification techniques, since each protein has a limited number of states and the genes regulating different proteins are assumed to segregate independently. Conversely, continuously distributed data such as morphometric traits, which are also frequently correlated, may not be suited to classification if the distributions for a trait in two populations are overlapped.

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Periodicity of Egg Laying and Egg Age Distributions in Planktonic Cladocera

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Tessier, A. J. 1984. Periodicity of egg laying and egg age distributions in planktonic Cladocera. Can. J. Fish. Aquat. Sci. 41: 409–413.

In natural populations of planktonic Cladocera, I observed significant diel, molt cycle, and cohort periodicity of egg laying. Diel cycles of egg laying were pronounced in one population of *Daphnia galeata mendotae*; most females laid eggs only at dawn. There was a strongly negative correlation between diel egg laying cycles of coexisting *Daphnia* species. Egg laying in two *Daphnia* populations cycled with a period approximating the egg development times, suggesting a synchronization of individual molt cycles. Noncontinuous reproduction was characteristic of a population of *Holopedium gibberum*. Adults reproduced synchronously and then died; there was little overlap of generations.

Dans des populations naturelles de Cladocera planctoniques, j'ai observé une périodicité significative dans la déposition des œufs, selon la période du jour, le cycle de mue et la cohorte. Les cycles nycthéméraux de déposition des œufs sont prononcés dans une population de *Daphnia galeata mendotae*; la plupart des femelles ne déposent leurs œufs qu'à l'aurore. Il y a forte corrélation négative entre les cycles de déposition des œufs d'espèces de *Daphnia* coexistantes. Dans deux populations de *Daphnia*, le cycle de déposition des œufs correspond à peu près à la période de développement des œufs, ce qui donne à penser que les cycles de mue individuels sont synchronisés. Une population de *Holopedium gibberum* est caractérisée par une reproduction discontinue. Les adultes se reproduisent synchroniquement et meurent ensuite ; il y a peu de chevauchement des générations.

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Most organisms time their reproduction to coincide with favorable environmental conditions. The greater the temporal inconsistency in habitat quality, the more critical becomes proper timing of reproduction. Among freshwater and marine zooplankton species, major reproductive periods are generally seasonal events. However, significant environmental fluctuation also occurs on smaller time scales and could presumably select for short-term reproductive periodicity. For example, many zooplankton populations vertically migrate and therefore expose themselves daily to drastic shifts in habitat quality (Threlkeld 1980). Nonmigrating populations may also experience diel changes in predation intensity, algal productivity, or even water chemistry (O'Brien and DeNoyelles 1972).

Harding et al. (1951) observed that marine planktonic *Calanus* confined egg laying to between 24:00 and 04:00, when adults were located in the upper waters as a consequence of vertical migration. Mullin (1968) confirmed this finding for *Calanus helgolandicus* (Claus) and showed that light cueing was important for egg release. In freshwater habitats, Saunders (1980) observed significant diel periodicity in egg eclosion and egg deposition rates in several, but not all, populations of rotifers in a tropical lake. Green (1956) found nonuniform egg age distributions in natural populations of *Daphnia magna* and *Daphnia pulex*. Intervals between apparent egg deposition suggest a synchronization of individual molt cycles. Brubaker (1972) provided evidence for diel periodicity of juvenile release in *Daphnia ambigua* and *Daphnia parvula* populations. Similar to marine copepods, the *Daphnia* tended to release young at night and in the surface waters.

Despite these suggestive findings, there are apparently no additional reports examining the generality of short-term reproductive periodicity in zooplankton populations, specifically, no investigations concerning diel or molt cycle periodicity of egg laying in cladocerans. Rather, most analyses of cladoceran population dynamics explicitly assume constant birth processes over intervals of several days to a week. My objective was to address the validity of that assumption by investigation three scales of reproductive periodicity in four populations of planktonic cladocerans inhabiting different lakes.

Methods

Holopedium gibberum in Lake Lacawac, Wayne City, PA, *Daphnia catawba* in Lake Giles, Pike City, PA, and *Daphnia galeata mendota* and *Daphnia pulex* in Lake Waynewood, Wayne City, PA, constitute the zooplankton populations and lakes I studied. All three lakes are located in the Pocono Mountains (alt. 420–440 m) and exhibit thermal stratification. Limnology of these lakes has been described by Goulden (1971), Phoenix (1976), and Tessier (1981).

Diel Periodicity

I examined diel periodicity in Caldocera egg laying on 10–12 July 1982 for *H. gibberum* and on 14–17 July 1982 for *D.*

galeata mendotae and *D. pulex*. On these respective dates the populations were sampled every 6 h (12:00, 18:00, 24:00, and 06:00). A sampling consisted of vertical hauls with a Wisconsin bucket net (13-cm diameter, 80-μm mesh) taken from lake bottom to top at a minimum of two different lake sites. Two hauls were made at each site and immediately composited into a single sample. Samples were preserved in cold, sugar–formalin (Prepas 1978). Collections from different lake sites were analyzed separately and considered as replicates.

I used an A/O zoom stereomicroscope to examine the animals and completed analysis within 3 h of collection. Greater than 50 but usually 100 females carrying eggs in egg stage 1 (30.2% of egg development time, Threlkeld 1979a) were picked randomly from each sample. Of these egg stage 1 clutches, the number of clutches containing eggs without transparent edges (4.5% of egg development time, Green 1956) were recorded. The ratio of number of clutches in the first 4.5% of development to number of clutches in the first 30.2% of development was calculated for each sample. Ratio mean and variance were estimated from replicate sites.

If diel egg recruitment and mortality are constant, the clutch age ratio will remain constant. If in addition, egg mortality is negligible, a uniform age distribution will result and the ratio of clutches in the first 4.5% of development to those in the first 30.2% of development will equal 0.15. Only the first third of the egg development was examined to minimize the impact of egg mortality. Since animals were exposed to an average temperature of greater than 20°C throughout July, egg development time was less than 72 h (Bottrell et al. 1965); hence, I used only clutches younger than 24 h (egg stage 1, Threlkeld 1979a).

Molt Cycle Periodicity

Daphnia galeata mendotae were sampled every 12–24 h on 15–19 August 1981, and *D. catawba* were sampled every 24–48 h on 10–18 June 1982. Generally, I visited a single site in each lake and composited three vertical hauls of a Wisconsin bucket net into a single sample. On 14 and 15 June 1982, three different sites were sampled and I analyzed collections from each site separately. Preservation of samples was as previously described. Between 50 and 200 fecund adults were examined on each date. Each clutch was categorized on the basis of egg morphology into one of five age groups (Threlkeld 1979a). The cumulative frequency distribution of clutches within the five age groups was compared with an expected, cumulative frequency distribution assuming constant egg recruitment and negligible egg mortality. Maximum deviation ($D_{\max}$) between expected and observed distributions was calculated for each sampling date (Kolmogorov–Smirnov test, Siegel 1956). If egg recruitment and mortality were constant, the $D_{\max}$ statistic would also remain constant over different sampling dates. As an additional, more intuitive measure of change in egg recruitment or mortality, the proportion of clutches in the first age category (30.2% of total egg development time) was also calculated.

Cohort Structure

The demography and reproductive activity of *H. gibberum* were monitored during July and August 1980. Sampling was conducted every 3–4 d. A single vertical haul of a Wisconsin bucket net was made at each of five lake sites. Samples were composited into a single sample and preserved as previously described. A minimum of 100 animals were randomly chosen and measured for total body length, and clutch size, and qualitatively scored for lipid and ovary indices (Tessier and Goulden 1982) using an A/O zoon stereomicroscope.

Results

Diel Periodicity

Diel changes in the ratio of freshly laid clutches (4.5% of egg development) to total clutches in egg stage 1 (30.2% of egg development) for *H. gibberum*, *D. galeata mendotae*, and *D. pulex* are shown in Fig. 1. All three populations displayed evidence of diel periodicity in egg laying.

Holopedium gibberum increased egg laying activity throughout night, exceeding the expected clutch age ratio of 0.15 by dawn (0.17–0.21). Egg laying apparently decreased during the day; the ratio dropped to a low of 0.08. Throughout this sampling period, population abundance of *H. gibberum* was stable; the population was composed largely of adults having a low mean fecundity. Furthermore, no significant changes in the body size structure of the population were observed between day and night samples (A. J. Tessier, pers. obs.).

Diel fluctuation in clutch age ratio was also observed in the *Daphnia* populations. In both populations, a minimum ratio significantly lower than the 0.15 level was reached. Egg laying in *D. galeata mendotae* showed rapid changes; the clutch age ratio shifted from a minimum of 0.01–0.05 to a maximum of 0.5–0.7 in only 6 h (24:00–06:00).

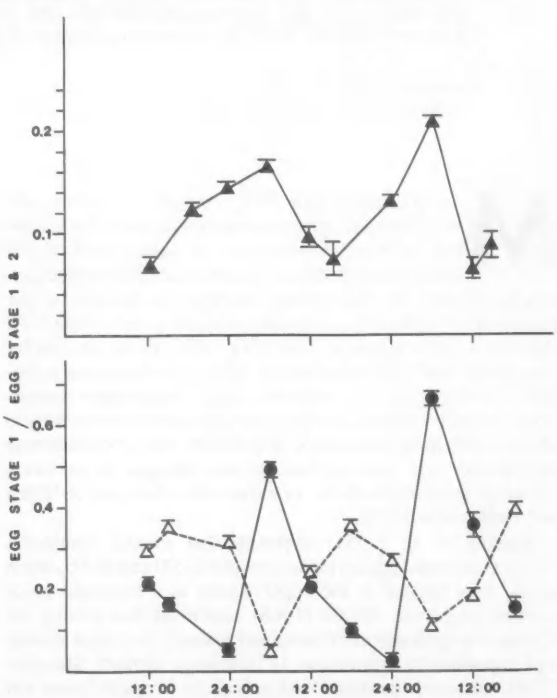

FIG. 1. Diel changes in the ratio of number of clutches in the first 4.5% of egg development (egg stage 1, Green 1956) to number of clutches in the first 30.2% of egg development (egg stages 1 and 2, Green 1956; Threlkeld 1979a). ▲, *H. gibberum* in Lake Lacawac; ●, *D. galeata mendotae* in Lake Waynewood; △, *D. pulex* in Lake Waynewood. Points represent mean ratio ± 1 SE. Expected ratio is 0.15 assuming constant egg recruitment and negligible mortality.

Diel fluctuation in clutch age ratio for *D. pulex* (0.05–0.35) was less extreme than for *D. galeata mendotae*, but interestingly, was out of phase. Maximal egg laying occurred at dawn for *D. galeata mendotae* whereas for *D. pulex* the ratio peaked at dusk. Similarly, a minimal ratio was apparent at dawn for *D. pulex* and at midnight for *D. galeata mendotae*. A significant negative correlation describes the inverse relationship of egg laying times for these two species in Lake Waynewood ($r_s = -0.77$, $P < 0.01$).

During this sampling period the population growth of the two *Daphnia* species was also different. *Daphnia galeata mendotae* abundance increased during July, whereas *D. pulex* abundance decreased. Similarly, changes in the clutch age $D_{\max}$ statistic reflected a shift toward dominance of younger clutches for *D. pulex* whereas *D. galeata mendotae* became dominated by older clutches.

Molt Cycle Periodicity

Apparent molt cycle periodicity was observed in the *D. catawba* and *D. galeata mendotae* populations during June 1982 and August 1981, respectively. Changes in the fraction of egg stage 1 clutches and in the clutch age $D_{\max}$ statistic are shown for both populations in Fig. 2. The *D. catawba* population shifted from 78% of all clutches in egg stage 1 to 27% and then back up to 50% in stage 1, all within a 5-d period. Changes in the $D_{\max}$ statistic reflect a similar pattern, changing from a dominance of young clutches ($D_{\max} = 0.48$, $P < 0.05$, Kolmogorov–Smirnov test) to a dominance of old clutches ($D_{\max} = -0.19$, $P < 0.5$) and then back to a dominance of young clutches ($D_{\max} = 0.25$, $P < 0.05$). Despite these radical shifts in egg laying, no significant changes were noted in the body size distribution of *D. catawba* between 10 and 15 June (Fig. 3; $P > 0.10$, Kolmogorov–Smirnov test).

A similar, though less dramatic cycle of egg laying was seen in *D. galeata mendotae*. The proportion of egg stage 1 clutches shift from 60 to 25% within a 3-d period and then increase to 54% by the 4th d. The $D_{\max}$ statistic traces a similar course from positive (dominance of young clutches) to negative (dominance of old clutches) and back to positive values again.

Interestingly, as the fraction of clutches in egg stage 1 approaches 0.3, the $D_{\max}$ statistic approaches zero (i.e. a uniform clutch age distribution is approached). Furthermore, the apparent 5-d cycle period of *D. catawba* at 15°C and 3-d cycle period for *D. galeata mendotae* at 22°C approximates the expected egg development times at those temperatures (Bottrell et al. 1976).

Cohort Periodicity

The entire population of *H. gibberum* in Lake Lacawac reproduced synchronously during midsummer 1980. Changes in the animal's mean clutch size and mean lipid and ovary indices during July and August 1980 are shown in Fig. 4. The proportion of total adults carrying eggs on each sampling date is also given. Essentially no reproduction occurred in July or early August. However, body lipid accumulated throughout this period, peaked in early August, and was transferred to the ovaries for egg production. Reproduction began in mid-August, peaked for a 2-wk period in late August, and then rapidly decreased.

Synchronous reproduction by *H. gibberum* was associated with a discrete cohort structure. Body size distribution of the *H. gibberum* population throughout July and August 1980 is presented in Fig. 5. Even before mid-July, the population consisted entirely of large adults. Short-term food addition experiments showed that these animals were reproductively mature at this time but lacked necessary reserves for reproduction (Tessier 1981). Individuals continued to grow until

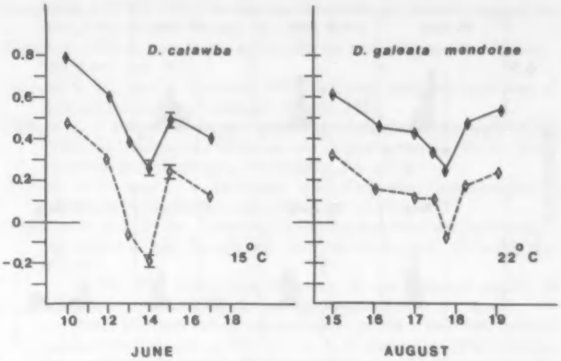

FIG. 2. Changes in the fraction of clutches containing eggs in the first 30.2% of egg development (◆) and in the maximum deviation between observed and expected cumulative frequency distributions of clutch age (◇). Mean ± 1 SE calculated for values on 14 and 15 June 1982. *Daphnia catawba* from Lake Giles in June 1982, mean water temperature of lake = 15°C. *Daphnia galeata mendotae* from Lake Waynewood in August 1981, mean water temperature in lake (surface to depth of water having oxygen concentration greater than 1 mg/L) = 22°C.

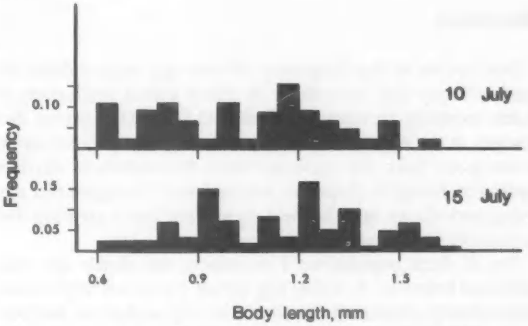

FIG. 3. Body size structure of the *D. catawba* population in Lake Giles during the sampling period investigating molt cycle periodicity. Body sizes measured from top of head to base of tail spine.

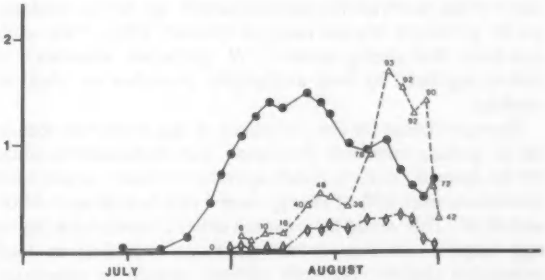

FIG. 4. Changes in the mean lipid index (●), mean ovary index (◇), and number of eggs per total number of females (△) in the *H. gibberum* populations in Lake Lacawac, 1980. Numbers next to points represent percentage of adults in the population carrying eggs.

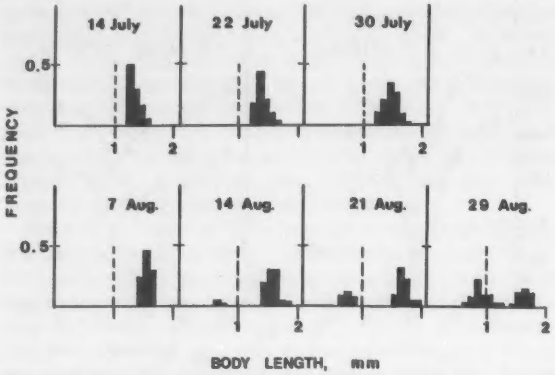

FIG. 5. Body length frequency distribution for *H. glbberum* in Lake Lacawac, 1980. Broken line at 1 mm represents approximate size at maturity.

mid-August, when the new cohort of animals began appearing and eventually replaced the parental cohort, which died in late August.

Discussion

Diel cycles in the frequency of one egg stage relative to another imply diel periodicity in either clutch production or clutch mortality (predation). Threlkeld (1979b) observed diel changes in the egg age distribution of *Daphnia* populations in Wintergreen Lake. He attributed these fluctuations to daytime feeding by bluegills (*Lepomis macrochirus*). I suggest that egg laying periodicity can also be a significant factor creating diel fluctuation in egg age distributions.

For all three populations I examined, the clutch age ratio decreased below 0.15. Either egg laying fluctuated or predators preferentially consumed clutches laid only within the last few hours. Although possible, the latter appears to be unlikely, as little gross morphological change accompanies the first 30% of egg development (Green 1956).

Field evidence that diel shifts in predation intensity are not the cause of the observed fluctuation in clutch age ratio is available for *H. gibberum* in Lake Lacawac (Tessier 1981). This work concludes that during summer, *H. gibberum* abundance is mainly regulated by food availability; predation on adults is minimal.

Strong evidence for diel periodicity of egg laying was seen in the *D. galeata mendotae* population. For predation to account for the dramatic shifts in clutch age ratio, predators would need to consume over 90% of the egg stage 1 clutches between 24:00 and 06:00. This would have caused either a relative scarcity of egg stage 1 clutches or an enormous reduction in total population density. No such obvious change in population density or abundance of individuals carrying egg stage 1 clutches was noted. Rather, analysis of the 06:00 samples proceeded quickly, an increase in absolute number of freshly laid clutches being more apparent than any decrease in number of egg stage 1 clutches. Furthermore, the egg age distribution for *D. galeata mendotae* during late July was dominated by older eggs, suggesting that vertebrate predation was not intense (mean $D_{\max}$ ± 1 SE for 20–30 July 1982 = -0.19 ± 0.02, $P < 0.05$, Kolmogorov–Smirnov test).

The concordance of these findings with those of Harding et al. (1951), Mullin (1968), and Saunders (1980) suggests that diel cycles of egg laying may be widespread among zooplankton. However, the observation of a negative correlation between egg laying cycles of *D. pulex* and *D. galeata mendotae* in the same lake suggests that more than one causal factor may be operating.

Interpretation of the apparent molt cycle periodicity exhibited by *D. catawba* in Lake Giles and *D. galeata mendotae* in Lake Waynewood is clouded. Observation of a second *D. catawba* population (Lake Waynewood) showed no evidence of simple molt cycle periodicity. However, a great deal of fluctuation in the clutch age distribution was evident (A. J. Tessier, unpubl. data). Since no significant shift in body size distribution was observed for *D. catawba* in Lake Giles, and since the clutch age distribution ($D_{\max}$) shifted from positive to negative and back to positive again, it appears likely that some periodicity with respect to molt cycle was occurring.

Tightly synchronous reproduction is extremely unusual for cladoceran populations. Yet the discrete cohort structure and synchronous reproductive behavior described for *H. gibberum* was typical of its summer population dynamics in 1979, 1980, 1981, 1982, and 1983 (A. J. Tessier, unpubl. data). In Lake Lacawac the entire *H. gibberum* population is recruited each spring from a hatch of resting eggs. This initial synchronization, coupled with extreme food limitation for this population (Tessier 1981), may explain the pattern. A long period of time was needed to gather enough resources to produce a clutch. The average clutch never exceeded two eggs, and animals died shortly after reproducing.

The recognition of egg laying periodicity on various time scales is of fundamental significance to an understanding of zooplankton dynamics. All conceptual models and analytical techniques assume constant birth (and death) rates over time intervals much longer than a few hours. Reinterpretation of data sets analyzed with this assumption of constancy may be required. For example, egg laying periodicity imposes severe limitations on the use of egg ratio or egg aging data. Taylor and Slatkin (1981) warned that temporal fluctuations in birth rates negate the suitability of egg ratio techniques for estimating population birth rates. Similarly, using egg age distributions to interpret changes in reproductive rates or mortality (Threlkeld 1979a, 1979b) will require short-interval sampling at precisely the same time of day over long periods (Gophen 1978). Still, changes in the amplitude of diel cycles of birth and death could go unnoticed. Alternatively, use of egg aging criteria to study egg laying periodicity may provide insights into ultimate factors regulating zooplankton populations. For example, how does diel egg laying relate to vertical migration, food availability, and the intensity and type of predation?

Acknowledgments

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An Evaluation of Morphometrics and Meristics for Stock Separation of Pacific Herring (*Clupea harengus pallasi*)

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Meng, H. J., and M. Stocker. 1984. An evaluation of morphometrics and meristics for stock separation of Pacific herring (*Clupea harengus pallasi*). Can. J. Fish. Aquat. Sci. 41: 414–422.

We conducted an analysis to determine if Pacific herring (*Clupea harengus pallasi*) stocks occurring in different localities in British Columbia waters could be separated using morphometric and meristic characters. Discriminant function analysis was applied to morphometric and meristic characters taken from food herring samples. Herring found in northern British Columbia waters were detectably different from those found in the Strait of Georgia. We recommend using meristic characters for separation on a broad geographic scale and using "best" morphometric characters for finer resolution within the established broader groups. We defined a set of 12 best morphometric characters for further large-scale studies.

Nous avons mené une analyse en vue de déterminer s'il était possible de séparer les stocks de harengs du Pacifique (*Clupea harengus pallasi*) de différents endroits des eaux de la Colombie-Britannique d'après leurs caractéristiques numériques et morphométriques. Des échantillons de harengs prélevés de pêches pour l'alimentation ont servi à une analyse de fonction discriminante appliquée aux caractères morphométriques et méristiques. On peut détecter des différences entre le hareng du nord de la Colombie-Britannique et celui du détroit de Géorgie. Nous recommandons de se servir des caractères méristiques pour une séparation préliminaire à grande échelle géographique, utilisant les « meilleurs » caractères morphométriques pour une définition plus précise à l'intérieur de ces groupes généraux. Nous définissons les 12 « meilleurs » caractères morphométriques pouvant être utilisés dans des études à grande échelle.

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It is important to management of British Columbia Pacific herring (*Clupea harengus pallasi*) to understand the significance and distribution of stocks. The concept of "stock," as we use it, follows the operational definition (Gulland 1969). A stock is a separate reproducing population or subpopulation of a species. If characters can be found to identify the separate reproducing populations of a species, then they can be modeled and managed because they have homogeneous production parameters. We use morphometric and meristic characters to improve our understanding of British Columbia herring stocks.

Historically, Tester (1949) identified at least six populations off the British Columbia coast from examining vertebral counts. Subsequently, tag recoveries led to the definition of nine so-called migratory populations (Taylor 1964). As the fishery changed from a reduction fishery to a roe fishery, and moved to the spawning grounds, the operational stock was defined as a management unit (Hourston 1980).

Two methods have recently been assessed in separating populations of Pacific herring. Electrophoresis was employed by W. S. Grant (Sea Fisheries Institute, Private Bag X2, Roggebaai, Cape Town, S. Africa, pers. comm.) to examine biochemical variation. Arthur and Arai (1980) employed a study of parasite distribution to separate stocks. Even though significant results were obtained from these studies, the methods are too laborious to establish operational definitions of stocks for British Columbia herring.

A more practical method for stock separation appears to be the use of morphometrics and/or meristics. Meristic variation in beaked redfishes (*Sebastes mentella* and *S. fasciatus*) has recently been investigated in the Northwest Atlantic (Ni 1982). Parson (1973) found a number of discrete Atlantic herring (*C. harengus harengus*) stocks in Newfoundland using meristics, and Schweigert (1981) used a limited set of morphometric and meristic characters on herring data from three locations on the Pacific coast of North America.

This study constitutes a first step in a series of investigations for stock separation of British Columbia herring. We use morphometric and meristic characters to evaluate their potential as stock separators for samples taken during the annual food herring fishery on migratory populations. The purpose of this study was (1) to examine morphometric and meristic data for evidence of northern and southern stock discreteness and (2) to find types of characters and the optimal number of characters required for stock separation.

Materials and Methods

Samples

Six samples were taken from commercial purse seiners fishing in five different localities in November 1981 (Table 1). Each sample consisted of 100 randomly selected individuals. Samples 1, 2, and 3 came from the Strait of Georgia (49°06′N, 123°45′W), whereas samples 4, 5, and 6 came from the North

TABLE 1. Herring food fishery samples taken in the Strait of Georgia (Div. 5) and the North Coast (Div. 2) (codes according to Hourston and Hamer 1979).

Sample No.	Division	Management unit	Section	Locality	Date
1	5	23	173	954 Boat Harbor	17 Nov. 1981
2	5	23	173	941 Yellow Point	17 Nov. 1981
3	5	23	173	950 Danger Reef	17 Nov. 1981
4	2	09	051	305 Bonilla Island	17 Nov. 1981
5	2	09	051	302 White Rocks	16 Nov. 1981
6	2	09	051	302 White Rocks	16 Nov. 1981

Coast (53°40′N, 129°30′W). Samples were deep-frozen as soon as possible and stored for further processing in the laboratory.

Morphometrics and Meristics

The fish were weighed and sexed and scales were removed for age determination. A set of 35 characters was examined, consisting of 27 morphometric characters and 8 meristic characters (Table 2; Fig. 1). For a detailed description of the methods of measurement the reader is referred to Hubbs and Lagler (1967). To reduce measuring errors, morphometric measurements were made by one person because differences of shape between samples were expected to be small. Measurements were taken to the nearest 0.1 mm using calipers and a ruler, except for fin ray heights, which were measured to the nearest 0.5 mm. To assess the effect of measurement errors, the set of 27 morphometric measurements was repeated 100 times on a separate single specimen.

Counts of vertebrae including urostyle were made from X-ray plates (DuPont Cronex Hi-Plus UI film). Fin ray counts and gill raker counts were made using a binocular dissecting microscope. Fin ray counts included all detectable rays counted at the base. Careful consideration was also given to the counting of dorsal and anal fin rays. We found, as did others (e.g. Copeman 1977), that accurate counting of fin rays was not possible from X-ray plates.

A variety of statistical approaches are used to correct for length effects in stock separation studies using differences in fish shape. Ihssen et al. (1981) and Schweigert (1981) used a log-linear regression to correct for length. In other studies, body measurements are expressed as ratios of fork length to remove variations due to fish size (e.g. Casselman et al. 1981; Copeman 1977). For the case in which differences in growth are considered too, only fish with the same age and life history are used and no correction for length is required (Copeman 1977). We found morphometric characters linearly related to standard length and thus used a linear GM regression (Ricker 1973) to correct for length:

$$\mathrm{MN} = \mathrm{V}(\bar{\mathrm{L}} - \mathrm{L}_\nu) + \mathrm{MO}$$

where MO = original measurement, MN = corrected measurement, $\bar{\mathrm{L}}$ = overall mean standard length, L_ν = standard length for individual fish, and V = GM regression coefficient within samples.

There was no need to correct the meristic characters for length, as these were independent of standard length in most cases. Only 4 of 36 regression coefficients showed a significant departure from zero ($P > 0.05$), and there was no apparent pattern concerning these departures.

A one-way analysis of variance (ANOVA) was applied to the corrected morphometric measures and original meristic counts to test for group differences. Levene's test for equal variances was performed using program BMDP7D (Dixon et al. 1981).

Discriminant function analysis was applied separately and combined to morphometric and meristic characters, using

TABLE 2. Morphometric and meristic characters and acronyms used for herring stock separation analysis.

Morphometric		
1	LENGTH	Standard length
2	DORCAUD	Insertion of dorsal fin to CMP[a]
3	PECCAUD	Insertion of pectoral fin to CMP
4	PELCAUD	Insertion of pelvic fin to CMP
5	ANALCAUD	Insertion of anal fin to CMP
6	SNDORS	Tip of snout to insertion of dorsal fin
7	PECTDORS	Insertion of pectoral fin to insertion of dorsal fin
8	PELDORS	Insertion of pelvic fin to insertion of dorsal fin
9	ANALDORS	Insertion of anal fin to insertion of dorsal fin
10	DORBASE	Base length of dorsal fin
11	ANALBASE	Base length of anal fin
12	DORSHEI	Dorsal fin height
13	ANALHEI	Anal fin height
14	PELVHEI	Pelvic fin height
15	PECTHEI	Pectoral fin height
16	PEDUNCLE	Peduncle height
17	SNOUTL	Snout length
18	CHEEKL	Distance between orbit and preopercle
19	HEADL	Head length
20	OCCIPL	Occipital length
21	INORBW	Inorbital width
22	SNOUTMAN	Mandible length
23	SNOUTMAX	Maxillary length
24	MAXW	Maxillary width
25	PECTW	Distance between pectoral fin insertions
26	PELVICW	Distance between pelvic fin insertions
27	HEADW	Head width
Meristic		
28	DORSFIN	Dorsal fin rays
29	ANALFIN	Anal fin rays
30	PELVFIN	Pelvic fin rays
31	PECTFIN	Pectoral fin rays
32	VERT	Vertebrae
33	GILLUP	Upper gill rakers
34	GILLOW	Lower gill rakers
35	GILLTOT	Total gill rakers

[a] CMP = caudal measuring point (Hourston and Miller 1980, p. 3).

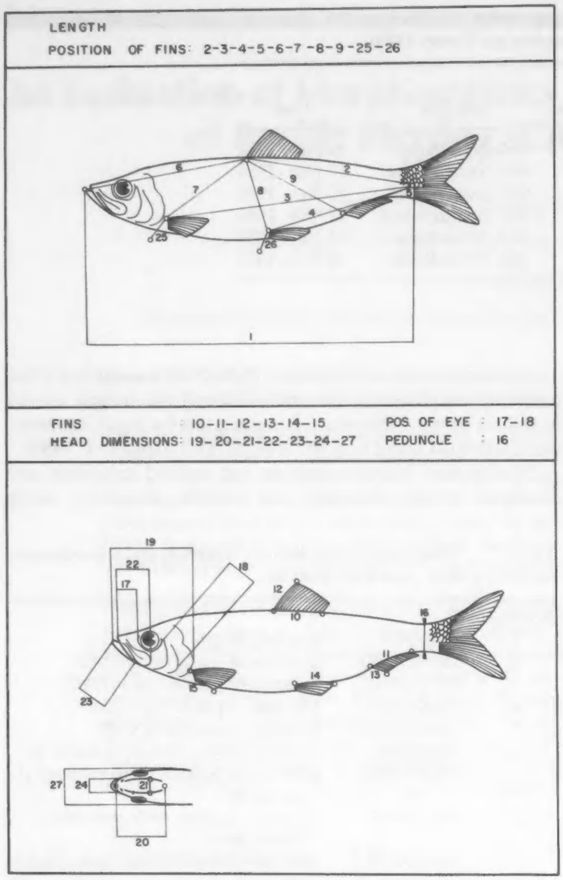

FIG. 1. Morphometric characters measured for herring stock separation analysis (for description of characters refer to Table 2).

BMDP7M (Dixon et al. 1981). The discriminant function was used to classify individual fish into groups employing the jackknife technique (for a discussion see Cook 1982). Different combinations of samples and sets of characters were run to (1) examine the relationship between samples and north and south groups and (2) to find a set of characters that consistently separates groups.

The minimum distance cluster analysis program (BMDP1M) (Dixon et al. 1981) was applied separately to matrices of Mahalanobis distances for morphometric measurements and meristic counts to illustrate the relationship between samples. Mahalanobis distance is a measure of the distance between the combined sample means (Morrison 1967).

Results

Differences between Samples Based on Morphometrics

The ANOVA on the adjusted morphometric measurements gave significant differences among samples at the 1% level for 23 measurements (Table 3). Only three measurements showed no significant differences at the 5% level.

The stepwise discriminant function analysis (SDFA) on the morphometric measurements gave significant differences among samples. Pairwise comparisons among the six samples were highly significant ($P \ll 0.001$) with the exception of the two White Rock samples whose comparison was only significant at the 0.25% level.

The classification function included 19 characters according to their discriminating power. The first four characters in order of their power of separation were PECTW, SNOUTMAX, MAXW, and HEADW. The jackknife classification matrix (Table 4) showed correct classification ranging from 42 to 67%, with an average of 55% (a priori probability = 0.167). Examination of the matrix reveals two relationships. First, there is similarity between the two White Rock samples, as already expressed by the relatively low F values in the pairwise comparison. Combining the two samples leads to correct classification into five groups ranging from 54 to 75%, with an average of 64% (a priori probability = 0.20). Second, there is a recognizable pattern suggesting combination of southern samples (Boat Harbor, Yellow Point, and Danger Reef) and northern samples (Bonilla and White Rock).

The jackknife classification technique revealed the 12 most powerful characters to be sufficient for separation (Fig. 2a). Inclusion of more than 12 characters does not improve separation in this case. This number was chosen to prevent losing information for separating stocks that occur in closer proximity.

In the scatter plot of all individuals on the first two canonical axes (Fig. 3) there is strong overlap of the White Rock samples (E, F), overlap of Bonilla (D) and White Rock samples, and overlap of Boat Harbor (A) and Yellow Point (B) samples. The first two canonical axes count for 59.3 and 20.4% of the total dispersion, respectively. There is also a clear north (D, E, and F) versus south (A, B, and C) separation on the first canonical axis.

The dendrogram (Fig. 4a) derived from cluster analysis based on 19 characters also shows a north versus south separation at a distance between the combined sample means of 5 (Mahlanobis D^2).

Differences between Samples Based on Meristics

The ANOVA on meristics showed significant differences among samples for six of the eight characters ($P \ll 0.001$) (Table 5). The mean counts of dorsal (DORSFIN) and pelvic (PELVFIN) fin rays are not significantly different for the six samples ($P = 0.05$). The pairwise comparison among the samples shows that the combined sample means within groups 1, 2, and 3 and 4, 5, and 6 are not different, respectively ($P > 0.1$). However, differences between each sample of the first group and the samples of the second group and vice versa are highly significant. The jackknife classification based on SDFA on meristics did not work well. Only 10–47% of fish were classified into the correct original group. The mean of 25% for the six samples was slightly higher than the expected value of 17%. This result is not surprising because the F values indicate a close relationship among samples 1, 2, and 3 and 4, 5, and 6, respectively. The four meristic characters in the classification function in order of importance were GILLOW, VERT, PECTFIN, and ANALFIN.

The canonical scatter plot of all points (fish) shows strong overlap of southern (A, B, and C) and northern (D, E, and F) samples (Fig. 3). The centers of the six groups lie mainly on canonical axis 1, and this axis accounts for 93% of the total dispersion.

The dendrogram (Fig. 4b) derived from cluster analysis based

TABLE 3. Morphometric measurements (means ± SD) and F values (ANOVA, df = 5,587) adjusted for standard length (203.7 ± 14.86 mm). **Significant at the 1% level; NS, not significant at the 5% level.

	Sample							
Variable	1 (n = 97)	2 (n = 97)	3 (n = 100)	4 (n = 100)	5 (n = 99)	6 (n = 100)	All (n = 593)	F
DORCAUD	103.5±1.8	103.8±2.2	103.5±2.3	102.7±2.0	102.7±2.1	102.7±1.9	103.1±2.1	5.7**
PECCAUD	159.4±2.2	158.5±2.5	159.0±2.3	159.2±2.0	160.4±1.9	159.8±2.5	159.4±2.2	7.8**
PELCAUD	94.4±2.7	92.9±2.6	93.4±2.5	92.5±2.3	93.0±2.3	92.4±2.5	93.1±2.5	9.2**
ANALCAUD	51.7±1.7	50.9±2.0	51.0±1.9	51.1±1.5	50.8±1.5	50.8±1.9	51.0±1.8	3.2**
SNDORS	106.1±2.1	105.4±2.1	104.4±2.4	105.4±2.2	105.8±2.1	106.0±2.0	105.5±2.2	8.1**
PECTDORS	71.6±2.0	70.3±2.2	69.5±1.9	70.0±2.0	70.5±2.1	70.1±1.8	70.3±2.0	12.0**
PELDORS	49.7±2.1	49.5±2.0	49.4±2.4	48.3±2.3	47.9±2.5	47.4±2.7	48.7±2.3	17.2**
ANALDORS	66.2±1.9	66.8±1.9	66.9±1.9	65.6±2.1	65.6±1.9	65.8±1.7	66.1±1.9	9.5**
DORSBASE	27.9±1.4	28.0±1.6	28.3±1.5	27.9±1.2	27.6±1.4	27.5±1.5	27.8±1.4	4.4**
ANALBASE	24.3±1.4	23.8±1.4	23.6±1.5	24.2±1.2	23.6±1.3	23.4±1.4	23.8±1.4	7.0**
DORSHEI	27.5±1.2	27.8±1.2	27.2±1.1	27.0±1.1	27.5±1.0	27.3±1.2	27.4±1.2	5.5**
ANALHEI	9.9±0.8	10.3±0.9	10.2±0.8	10.1±0.8	10.5±0.7	10.3±0.7	10.2±0.8	5.6**
PELVHEI	20.7±0.9	20.2±0.9	20.1±0.8	20.7±0.8	20.7±0.9	20.6±0.8	20.5±0.9	11.9**
PECTHEI	31.9±1.1	31.8±1.3	31.8±1.0	31.9±1.1	32.0±1.2	31.8±1.2	31.9±1.2	0.8(NS)
PEDUNCLE	17.8±0.6	17.7±0.6	17.7±0.7	17.3±0.6	17.1±0.6	17.1±0.6	17.5±0.6	28.6**
SNOUTL	13.3±0.5	13.1±0.5	13.2±0.5	13.3±0.5	13.5±0.6	13.2±0.6	13.3±0.5	6.4**
CHEEKL	16.4±0.6	16.4±0.7	16.3±0.6	16.3±0.5	16.2±0.6	16.0±0.7	16.2±0.6	5.2**
HEADL	50.3±1.0	50.3±1.0	50.4±0.9	50.7±1.2	50.7±1.3	50.6±1.5	50.5±1.2	2.0(NS)
OCCIPL	34.2±0.7	34.1±0.9	34.2±0.8	34.1±0.8	34.1±1.0	33.9±0.9	34.1±0.9	1.6(NS)
INORBW	7.0±0.4	6.9±0.5	6.9±0.4	7.0±0.4	7.1±0.4	7.0±0.4	7.0±0.4	3.7**
SNOUTMAN	23.5±0.6	23.6±0.7	23.8±0.7	23.9±0.7	23.8±0.7	23.5±1.0	23.7±0.7	4.5**
SNOUTMAX	26.8±0.6	26.7±0.7	26.9±0.7	27.2±0.7	27.3±0.8	27.1±1.0	27.0±0.7	9.2**
MAXW	11.0±0.5	11.2±0.6	10.8±0.4	10.6±0.4	10.7±0.5	10.6±0.4	10.8±0.5	27.7**
PECTW	14.0±0.9	14.2±0.9	13.7±0.8	12.9±0.8	13.3±0.8	13.0±0.7	13.5±0.8	41.1**
PELVICW	8.3±0.4	8.2±0.4	8.2±0.4	8.0±0.4	8.0±0.4	8.0±0.4	8.1±0.4	9.0**
HEADW	19.7±0.6	19.6±0.6	19.9±0.6	19.3±0.6	19.3±0.7	19.0±0.7	19.5±0.6	24.0**

TABLE 4. Jackknifed classification: 6 groups and 19 characters.

		No. of fish classified into group					
Group	% correct	Boat-Hbr	Yellow-P	Danger-R	Bonilla	White-R5	White-R6
Boat-HBr	58.8	57	16	7	8	5	4
Yellow-P	67.0	10	65	14	4	1	3
Danger-R	65.0	7	15	65	10	1	2
Bonilla	54.0	6	5	10	54	11	14
White-R5	45.5	6	2	1	11	45	34
White-R6	42.0	6	2	1	20	29	42
Total	55.3	92	105	98	107	92	99

TABLE 5. Meristic counts (means ± SD) and F values (ANOVA, df = 5,585). **Significant at the 1% level; NS = non-significant at the 5% level.

	Sample							
Variable	1 (n = 94)	2 (n = 98)	3 (n = 99)	4 (n = 100)	5 (n = 100)	6 (n = 100)	All (n = 591)	F
DORSFIN	18.4±0.7	18.5±0.6	18.5±0.6	18.5±0.6	18.5±0.7	18.5±0.6	18.5±0.6	0.3(NS)
ANALFIN	16.3±0.9	16.3±0.9	16.4±0.8	16.8±0.7	16.5±0.8	16.5±0.8	16.5±0.8	5.2**
PELVFIN	9.0±0.1	9.0±0.2	9.0±0.1	9.0±0.2	9.0±0.1	9.0±0.0	9.0±0.1	1.0(NS)
PECTFIN	17.6±0.8	17.4±0.8	17.5±0.7	17.2±0.6	17.2±0.7	17.3±0.8	17.4±0.7	5.3**
VERT	52.7±0.7	52.7±0.7	52.7±0.7	53.1±0.6	53.1±0.7	53.1±0.7	52.9±0.7	10.3**
GILLUP	19.5±1.0	19.5±0.9	19.5±0.8	20.1±1.0	20.0±0.8	19.9±0.9	19.8±0.9	7.5**
GILLOW	43.8±1.4	44.0±1.5	44.3±1.2	45.1±1.6	45.1±1.4	45.1±1.6	44.6±1.5	15.8**
GILLTOT	63.3±2.0	63.5±2.1	63.9±1.7	65.1±2.3	65.1±1.9	64.9±2.2	64.3±2.0	16.1**

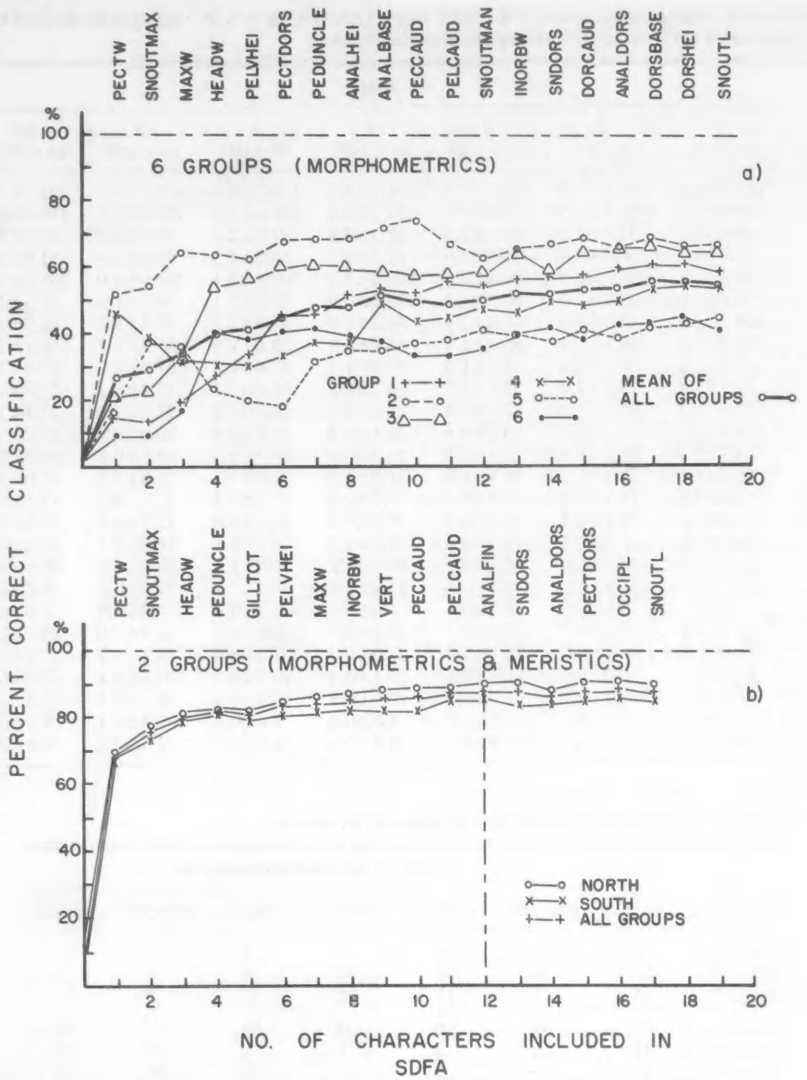

FIG. 2. Percentage of individuals correctly classified versus number of characters included in SDFA for (a) differences between samples based on morphometrics and (b) differences between north and south based on morphometrics and meristics.

on four meristic characters shows qualitatively the same relationship among samples as in Fig. 4a. However, the relative distance between the level of linkage of group north and south, and within each group, is much larger.

Separation of North from South

Differences between the original six samples showed that there is a clear north versus south pattern. Based on these results, SDFAs were applied to combined northern and southern samples for (1) 27 morphometric characters, (2) 8 meristic characters, and (3) all 35 characters (Table 6). The maximal separation success was 85 and 90% for south and north, respectively, using 17 characters (14 morphometrics and 3 meristics). The first two characters entered in the classification were the same as those emerging from six samples, namely PECTW and SNOUTMAX. PECTW accounted for 70% of the separation success, whereas SNOUTMAX and HEADW accounted for 7.7 and 4.3%, respectively (Fig. 2b). After step 11, there is no significant improvement of classification success.

The histogram of the canonical variable (Fig. 5) shows a relatively good separation into two groups, although there is some overlap of the two distributions.

Set of "Good" Morphometric Characters

To be useful for separating groups, two categories of properties are associated with "good" characters. First, the

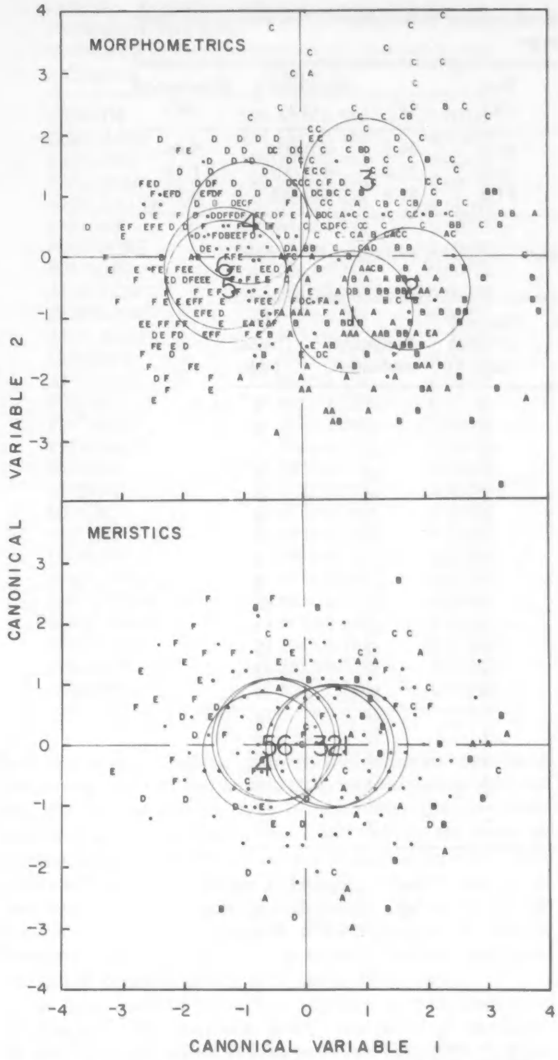

FIG. 3. Scatter plots from SDFA on morphometric measurements and meristic counts for six food herring samples.

character has a highly significant *F* value in ANOVA, which means it has high separating power in SDFA. Second, characters should have well-defined and easily reproducible measures. For this study, the overall ranking of 20 morphometric characters is shown in Table 7. The rank of the character is a combined measure of the two categories of properties mentioned above. Columns 2 and 3 of Table 7 depict the steps at which each character was entered into the classification function for two and six groups, respectively. Column 4 ranks the characters on the basis of the coefficient of variance obtained from repeated measurements (Table 8) into four classes. The subjectively assigned ranks of good (G), intermediate (M), and bad (B) shown in column 5 are based on the experience of measuring them.

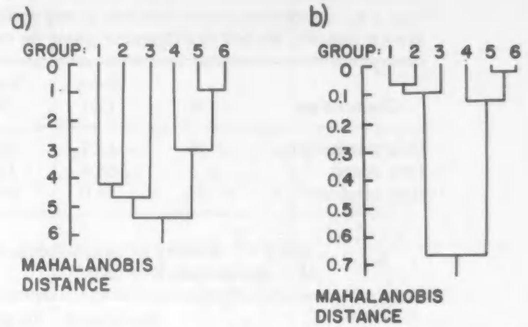

FIG. 4. Dendrogram derived from cluster analysis of Mahalanobis distances for (a) 19 morphometric measurements and (b) 4 meristic counts.

Results from jackknifed classifications (Fig. 2) indicated 12 characters to be optimal for separation. We define the first 12 characters of Table 7 as the set of "good" characters.

According to these results, pectoral width (PECTW) is the most important character, followed by head measures (SNOUTMAX, HEADW, MAXW), pelvic fin height (PELVHEI), peduncle height (PEDUNCLE), and position of fins (DORCAUD, PECCAUD, PECTDORS, PELCAUD).

To test whether all the important information is included in the set of 12 good characters we ran SDFA using all morphometric characters for the southern and northern samples separately. In the south, the first characters included in the classification function were members of the set of good characters. Relatively good separation between these nearby locations was obtained (Fig. 6). In the north, the first character entered in the classification function was a so-called bad character (ANALBASE), but four of the first seven characters were members of the set of good characters. Considerable overlapping of the three samples occurred, indicating biological similarity or stock intermingling in the three northern samples (Fig. 6).

Discussion

An objective of this investigation was to select the "best" set of characters for a similar study on a much larger scale. As discussed in Pella and Robertson (1979), the success of SDFA depends on the quality of the learning set. That means the learning set should represent a well-defined stock in the best possible way. Because herring migrate during their life from feeding grounds to spawning grounds and vice versa, and at the same time stocks mix and separate to some extent, the life history is a dynamic process. The samples were taken from herring on their migration to spawning grounds, and therefore possibly from a mixture of spawning stocks. This is considered in the following discussion and interpretation of the results.

We were able to maintain measurement error between 0.9 and 12.6% of the total variance. The change of measures due to keeping fish in the deep-freezer for varying periods was negligible, particularly since all morphometric measurements were corrected for standard length. After all measurements were taken, the first five fish were measured again and results were compared with the original measurements. We found that most of the characters differed by less than 2.5%. this result was

TABLE 6. Jackknifed classification into groups north and south, number of characters (N) included in the classification function, and test of differences among the two groups.

Character set	N	South (%)	North (%)	Total (%)	F	Significance (P)	Mahalanobis D^2
Only morphometric	16	83.7	88.0	85.8	49.1	$\ll 0.001$	5.46
Only counts	5	68.4	72.7	70.6	33.9	$\ll 0.001$	1.17
Both combined	17	85.0	90.0	87.5	51.8	$\ll 0.001$	6.18

TABLE 7. Ranking of morphometric characters (subjective evaluation: G = good; M = intermediate; B = bad).

Character	North/south character rank	Six groups character rank	Range of C values (Table 8)	Subjective evaluation	Overall rank
PECTW	1	1	3	G	1
SNOUTMAX	2	2	2	G	2
HEADW	3	4	2	G	3
MAXW	6	3	3	M	4
PELVHEI	5	5	3	M	5
PEDUNCLE	4	7	3	G	6
DORCAUD	12	15	1	G	7
PECCAUD	8	10	1	G	8
PECTDORS	11	6	2	G	9
PELCAUD	9	11	2	G	10
INORBW	7	13	2	G	11
SNDORS	10	14	1	G	12
ANALBASE	20	9	3	B	13
ANALHEI	20	8	4	B	14
CHEEKL	13	20	2	G	15
HEADL	14	20	1	G	16
SNOUTMAN	20	12	3	M	17
OCCIPL	15	20	1	G	18
SNOUTL	16	19	2	G	19
ANALDORS	20	16	2	B	20

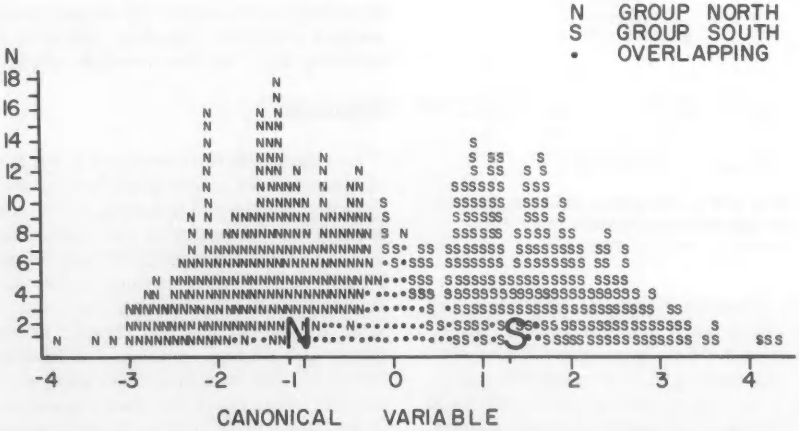

FIG. 5. Histogram of the canonical variable showing separation into northern and southern groups.

included in the ranking of characters as good, medium, and bad (Table 7).

All morphometrics showed a linear relationship between the character and standard length. Therefore, it was appropriate to correct according to the equation we used for differences in standard length and thus to remove the length effect. With this procedure we look only for differences in shape, but we lose, of course, all information connected with the age–length characteristics of different stocks. The SDFA on age-classes (not corrected for different standard lengths) resulted in a much better classification success with much smaller samples. The reason for this could be that an age-class of a sample could be more homogenous with regard to the life history and thus represent a better "learning set" for SDFA. Although a lot of

TABLE 8. Repeated morphological measurements (*C* = coefficient of variation).

Character	Mean ± SD	*C*
LENGTH	199.550±0.359	0.00180
DORCAUD	101.334±0.428	0.00423
PECCAUD	155.297±0.701	0.00451
PELCAUD	89.077±0.676	0.00759
ANALCAUD	51.379±0.559	0.01087
SNDORS	103.644±0.275	0.00265
PECTDORS	66.453±0.418	0.00630
PELDORS	47.298±0.341	0.00722
ANALDORS	63.864±0.529	0.00829
DORSBASE	26.448±0.205	0.00774
ANALBASE	20.977±0.337	0.01608
DORSHEI	26.650±0.314	0.01178
ANALHEI	9.665±0.236	0.02445
PELHEI	19.965±0.228	0.01140
PECTHEI	31.511±0.198	0.00630
PEDUNCLE	17.194±0.185	0.01074
SNOUTL	13.300±0.115	0.00868
CHEEKL	15.910±0.120	0.00755
HEADL	49.493±0.228	0.00462
OCCIPL	33.546±0.102	0.00304
INORBW	6.278±0.042	0.00663
SNOUTMAN	23.167±0.262	0.01130
SNOUTMAX	26.662±0.231	0.00866
MAXWBASE	10.518±0.168	0.01601
PECTBASE	12.671±0.181	0.01425
PELVICW	7.561±0.127	0.01680
HEADW	18.550±0.160	0.00865

work was done by "ageing" personnel on scales and otoliths on our herring, we still had large uncertainty about true ages and thus did not incorporate ages in our study. But all results indicate that the age–length data could increase the power of morphometric measurements as a tool of stock separation. Since counts are independent of age and length after age 3, there is no need to correct for standard length or age. Therefore, all fish from a sample can be used in stock identification studies. Furthermore, meristic counts represent the genetic configuration of fish better than measures (Ihssen et al. 1981).

The labor required is roughly proportional to the number of morphometric measurements taken. The set of 12 characters includes as much information as possible. The SDFA on these 12 characters, instead of all 17, reduced the jackknifed classification success only from 87.5 to 86.1% (Fig. 2b) for classification into north and south. Also, using 12 characters instead of all 19 reduced the jackknifed classification success from 55.3 to 49.1% (Fig. 2a) for classification into six groups. We also regarded the power of separation of nearby samples (Fig. 6) and found all the important characters to be included in the set of good characters. We consider the set of 12 characters as optimal for a study on a larger scale based on morphometric measurements, since no characters that are potentially difficult to measure (B in Table 7) are included in the set.

A comparison of the two dendograms (Fig. 4) based on morphometrics and meristics, respectively, shows the same general pattern, but the relative distance between the samples is very different. Considering the relative level of linking north (samples 4, 5, and 6) and south (samples 1, 2, and 3) with respect to each subcluster as a useful characteristic, the set of counts is a much more powerful tool for separation of stocks.

All SDFAs showed varying amounts of overlapping of samples or groups of samples. Overlapping of nearby samples (1, 2, and 3, and 4, 5, and 6, respectively) might be either an effect introduced by using samples from the food fishery and could be lowered by using samples from the roe fishery or an expression of mixing of different subpopulations. Overlapping between north and south (Fig. 5) may be an expression of exchange between these two geographical regions.

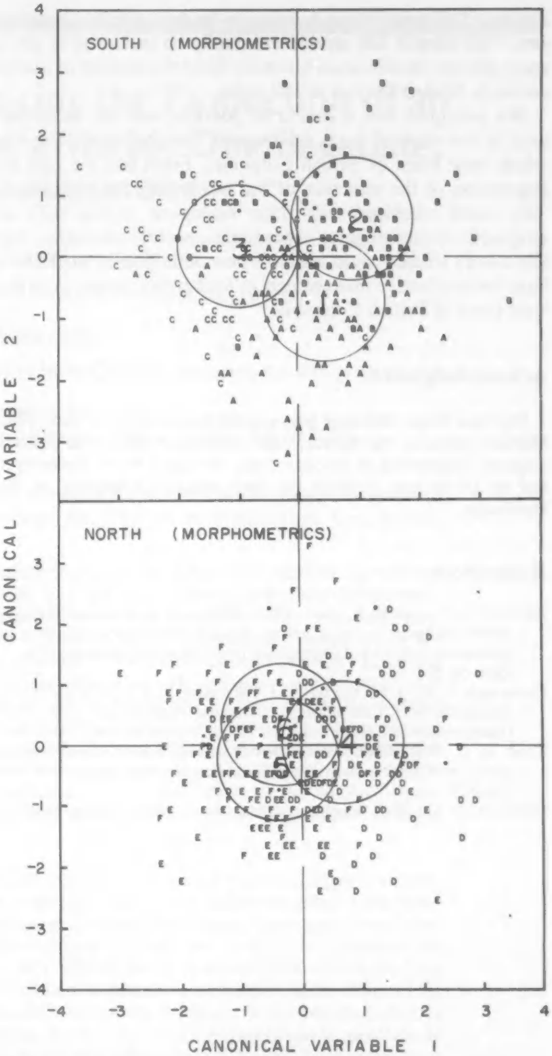

FIG. 6. Scatter plots from SDFA on morphometric measurements for the northern and southern food herring samples.

From the morphometric and meristic examination of food fishery samples a consistent conclusion is that British Columbia herring found in the north and in the south are detectably different. We recommend the use of meristic characters for separation on a broad geographical scale with intense sampling effort. However, for finer resolution within a major established group, we recommend the use of best morphometric characters for separation. For further studies, samples should be taken during the roe fishery at a time when there is a minimum of mixing of subpopulations to provide the best possible learning sets. The sample size should eventually be increased to get a more precise classification function, since the number of useful counts is limited (to four in our case).

We anticipate that it should be possible with the technique used in our study to get a dendrogram stretched out along the whole west coast of British Columbia. From this we gain an impression of the structure of the whole herring population. This result combined with other biological studies such as enzymatic studies, tagging experiments, ecological studies, and life history studies could lead to a new definition of stocks as a base for an effective management of the herring resources on the west coast of British Columbia.

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Environmental Factors Influencing the Production of an Antibacterial Metabolite from a Marine Dinoflagellate, *Prorocentrum minimum*

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Prorocentrum minimum, an ubiquitous marine dinoflagellate that does not form toxic red tides, produces biologically active extracellular metabolites, one of which is 1-(2,6,6-trimethyl-4-hydroxycyclohexenyl)-1,3-butanedione, a norcarotenoid referred to herein as β-diketone. The β-diketone was released extracellularly in a single "pulse" during the stationary phase of growth, and irradiance and temperature are important in controlling its maximum extracellular concentration. Different concentrations were obtained depending on the limiting nutrient. If cells were P deficient, the final extracellular concentration was twice that of N-deficient cultures. Iron-deficient growth further reduced the amount of the β-diketone produced. The ambient nitrate concentration in the medium had a strong influence on the maximum amount of the β-diketone produced. None of the environmental factors affected the temporal pattern of production. That the β-diketone is an antibiotic metabolite active against marine bacteria is shown by the reduction of the growth rate of three of four marine isolates and the inhibition of the heterotrophic uptake of glucose. The β-diketone in the natural environment may serve to reduce degradation of algae by physically associated bacteria.

Prorocentrum minimum, un dinoflagellé marin omniprésent qui ne cause pas de marées rouges toxiques, produit des métabolites extra-cellulaires biologiquement actifs, dont un est le 1-(2,6,6-triméthyl-4-hydroxycyclohexenyl)-1,3-butanedione, un nor-caroténoïde désigné ici comme β-dicétone. Cette substance a été mise en liberté hors des cellules en une seule « impulsion » durant la phase stationnaire de croissance, et l'irradiance et la température sont d'importants facteurs de contrôle de sa concentration extra-cellulaire maximale. Selon le nutriment limitatif, on obtient différentes concentrations extra-cellulaires. Quand les cellules ont une carence de phosphore, la concentration extra-cellulaire finale est le double de celle des cultures avec carence d'azote. Une croissance avec carence de fer réduit encore davantage la quantité de β-dicétone produite. La concentration des nitrates ambiants dans le milieu de culture influe fortement sur la quantité maximale de β-dicétone produite. Les modalités temporelles de production ne sont influencées par aucun des facteurs environnementaux. Le ralentissement de la croissance de trois des quatre isolats marins et l'inhibition de l'absorption hétérotrophe de glucose indiquent que la β-dicétone est un métabolite antibiotique actif contre les bactéries marines. La β-dicétone dans le milieu naturel peut servir à diminuer la dégradation des algues par des bactéries qui leur sont physiquement associées.

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Phytoplankton convert large amounts of their fixed carbon into extracellular metabolites. The majority of these metabolites are primarily organic compounds that play an important role in the heterotrophic cycle of aquatic ecosystems (Aaronson 1971; Hellebust 1974). A smaller amount of less well defined secondary, or unique, metabolites is also produced. Compounds in this latter group may be important in controlling interactions among phytoplankton and other organisms, or in regulating intracellular metabolism.

An example of these less well studied, unique metabolites is acrylic acid, first noted in *Phaeocystis pouchetti* by Sieburth (1959). Evidence by Guillard and Hellebust (1971) suggested that acrylic acid should exist extracellularly (2% of total excreted carbon) and they calculated external acrylic acid concentrations in natural blooms to be 7 $\mu g/L^{-1}$. Another unique extracellular metabolite involved in marine phytoplankton ecology is "aponin," base-labile organic substances produced by the cyanobacterium *Gomphosphaeria aponina*. At low concentrations this compound causes lysis of the marine dinoflagellate *Gymnodinium breve* (Martin and Martin 1976; Eng-Wilmot et al. 1979).

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Of interest is the production by phytoplankton of norcarotenoids, a group of organics traditionally defined as possible carotenoid breakdown products. Norcarotenoids are antifungal and antibacterial agents (Zajic and Kuehn 1961) and have been found in waters associated with blooms of freshwater cyanobacteria (Tabachek and Yurkowski 1976). Natural populations of *Microcystis wesenberg* produce the norcarotenoid that gives the bloom a tobacco-like odor (Jüttner 1979a). The fragrant, volatile nature of norcarotenoids enabled Jüttner to isolate four norcarotenoids produced extracellularly by *Cyanidium caldarium* (Rhodophyceae) under large-scale culturing conditions. These include β-ionone, geranylacetone, methylheptenone, and dihydrotrimethylnaphthalene (Jüttner 1979a).

The antibiotic nature of the norcarotenoids produced by *C. caldarium* has been established. Of the four norcarotenoids, β-ionone and dihydrotrimethylnaphthalene were inhibitory to cyanobacteria at 10 ppm ($10\,\mathrm{mg \cdot L^{-1}}$). Geranylacetone reduced growth of cyanobacteria at 50 ppm. Methylheptenone was the least inhibitory to the cyanobacteria examined, since concentrations greater than 200 ppm were required to reduce the growth of the assay species (Jüttner 1979b). The antibacterial nature of norcarotenoids in aquatic systems has also been examined by Reichardt (1981). Methylheptenone inhibited colonial growth of all bacterial species examined and was inhibitory to glucose uptake at 2 ppm ($18\,\mu\mathrm{mol \cdot L^{-1}}$). Conversely, β-ionone and geranylacetone inhibited only pigmented bacteria. The mechanism of inhibition involves the blockage of pigment production by the bacteria. This is a similar mechanism to the inhibition of carotenoid synthesis in the cyanobacterium *Synechococcus* by the addition of geranylacetone (Jüttner 1979b).

Recently, Andersen et al. (1980) characterized the extracellular norcarotenoid 1-(2,6,6-trimethyl-4-hydroxycyclohexenyl)-1,3-butanedione from laboratory cultures of the marine dinoflagellate *Prorocentrum minimum*. This compound, which we refer to herein as the β-diketone, is an example of an antibacterial norcarotenoid that imparts a tobacco-like odor to *P. minimum* cultures. The production of the β-diketone by *P. minimum* was examined by Trick et al. (1981). Production was dependent on the physiological state of the cells and was not simply a result of the photooxygenation of carotenoids. The β-diketone is antibacterial under both laboratory (Andersen et al. 1980) and field conditions (this paper). Understanding environmental factors that control the production and release of this metabolite may provide insight into its possible role in controlling the interactions between *Prorocentrum* species and associated microorganisms.

In this paper, the influence of environmental factors on the amount of β-diketone produced is examined. Factors such as irradiance, temperature, and salinity are considered for phosphate-deficient batch cultures. Because the β-diketone is a potential trace metal chelator (Andersen et al. 1980), production under iron-limited growth conditions was also investigated.

Materials and Methods

All *Prorocentrum* species were grown in enriched artificial seawater medium (Harrison et al. 1980) at 18°C with the exception of *P. mariae-lebouriae*, which required an enriched natural seawater-based medium of lower salinity (15‰) and elevated temperatures (22–24°C) for growth. Both media were modified by the replacement of sodium glycerophosphate with Na_2HPO_4 and $Fe(NH_4)_2SO_4$ with $FeCl_3$. Phosphate in the enrichment solution was reduced to $6\,\mu\mathrm{mol \cdot L^{-1}}$ (N:P = 27:1 by atoms) to ensure that the batch cultures became phosphate starved during senescence.

To achieve iron-starved growth, *P. minimum* was grown in the following media: (1) charcoal-treated natural seawater followed by the addition of all required nutrients (ESNW) (Harrison et al. 1980) and (2) charcoal-treated natural seawater followed by the removal of most of the available iron by the procedure of Lewin and Chen (1971) and then supplemented with all nutrients except $FeCl_3$ and EDTA (Morel et al. 1979). The first medium provided an iron-sufficient control, while the second medium provided iron-limited growth conditions. Vanadium was added as NH_4VO_3 to alleviate the effect of iron limitation (Meisch and Bielig 1975) on growth in one set of experiments.

Continuous lighting was provided by daylight fluorescent bulbs. The irradiance was reduced to 120, 90, 60, and $30\,\mu\mathrm{E \cdot m^{-2} \cdot s^{-1}}$ (Ei = einsteins) by wrapping cultures in neutral density screening in certain experiments.

Cells were grown axenically in 6- or 10-L flat-bottomed boiling flasks with constant stirring (60 rpm). The axenic nature of the cultures was verified visually using the acridine orange direct epifluorescent count technique (Hobbie et al. 1977) or by plating on an organic sterility medium and examining for growth after 48 h. If bacterial contamination was discovered, the cultures were discarded.

For some experiments, *P. minimum* was grown at N:P ratios ranging from 2 to 100. The atomic ration of N:P = 25 was chosen as the theoretical ratio where N and P were both limiting to growth, based on the disappearance of nitrate and phosphate from the batch culture medium. To achieve these experimental nutrient ratios without decreasing the final cell yield of the cultures, the amount of nonlimiting nutrient was increased in the medium. For example, N:P ratios greater than N:P = 25 (by atoms) were achieved by maintaining the limiting nutrient (P) at $6\,\mu\mathrm{mol \cdot L^{-1}}$ and increasing the amount of added N until the desired ratio was achieved.

Cultures were harvested and the β-diketone was collected using the procedure outlined by Trick et al. (1981). Briefly, cells were removed from the entire 6- or 10-L culture by continuous centrifugation. The remaining cells were removed by filtering the medium through a glass filter (GF/A), followed by a 0.45-μm membrane filter. The cell-free filtrate was acidified (pH 2.0) with HCl. Organics were removed from the acidified filtrate by passage over a 25 × 2 cm column of XAD-2 resin (Mallinckrodt). The XAD-2 resin collected a portion of the extracellular organics that included the β-diketone. The column was washed with 150 mL of H_2O and the organics were eluted with 300 mL of methanol. The methanol fraction was reduced to dryness by rotary evaporation and the residue was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was reduced to dryness and the amount of β-diketone in the crude fraction was determined using HPLC (Trick et al. 1981). Using this procedure, recovery efficiency of the β-diketone was 80%, and all reported values have been corrected accordingly.

Cell concentrations were determined using an electronic particle counter (Coulter Counter model TA-II), and changes in cell concentration with time were used to determine growth rates.

To establish the β-diketone as a biologically important metabolite, four marine bacterial species were chosen as bioassay organisms. *Pseudomonas acidivaris* and *Vibrio* sp. were obtained from the American Type Culture Collection (ATCC). *Flavobacterium* sp. and a *Chromobacterium* sp. were isolated from cultures of the marine dinoflagellate, *Scrippsilella swenneya*; taxonomic identification is tentative, based entirely on colony shape and color.

Bacterial cultures were maintained at 18°C in liquid broth cultures (ISOL medium) (Bell and Mitchell 1972). Growth was monitored by recording changes in absorbance of the culture at 750 nm. Inhibition experiments were similar to those of Chan et al. (1980). ISOL medium with 1.5% agar was made into plates and inoculated with a lawn of the bioassay species. A single 1-cm paper filter disc, which had been inoculated with 100 μg of the β-diketone and autoclaved, was placed on the agar in the center of the plate. Plates were incubated at 18°C and examined after 24 and 48 h. After 48 h the size of the zone of inhibition, which was seen as a clear zone around the disc, was recorded.

To examine the influence of known concentrations of the β-diketone on the growth of *Chromobacterium*, liquid ISOL medium cultures were employed.

The heterotrophic activity of natural bacterial populations was determined as follows. A subsurface sample was collected from two stations from the Fraser River plume in the Straight of Georgia, off Vancouver, B.C. (March 18, 1982). The sample was separated into 50-mL aliquots and different amounts of β-diketone were added. Two millilitres of [^{14}C]glucose (sp. act. = 4.43 $mCi\cdot mmol^{-1}$ (163.9 $MBq\cdot mmol^{-1}$)) was added and all samples were placed in running surface water (7.5°C). After 1 h of incubation in the dark, samples were filtered through 0.22-μm Nucleopore filters. The filters were washed with 10 mL of filltered (0.22 μm) seawater and placed in scintillation fluor. Counts were taken after 40 h using a Unilux III scintillation counter. Because bacterial counts were not taken, results are given as the percent inhibition compared with the control (no added β-diketone).

Results

Production under P-limited Growth Conditions

Of the six *Prorocentrum* species examined (Table 1), *P. minimum* var. *balticum*, *P. gracile*, and *P. maximum* showed no detectable extracellular β-diketone production and were not examined further. *Prorocentrum micans* and *P. mariae-lebouriae* had lower amounts of β-diketone production (10 $\mu g\cdot L^{-1}$) compared with *P. minimum* (40 $\mu g\cdot L^{-1}$) (Table 1), the original producer of the β-diketone material (Andersen et al. 1980). Small variations in the irradiance had a substantial influence on the amount of β-diketone produced by *P. minimum* (Fig. 1). A reduction in irradiance from 160 to 120 $\mu Ei\cdot m^{-2}\cdot s^{-1}$ reduced the amount of β-diketone produced by 50%, but it had no influence on the maximum growth rate. No β-diketone was produced at 60 $\mu Ei\cdot m^{-2}\cdot s^{-1}$, a level at which light became limiting to the growth of *P. minimum*.

While the absolute amount of β-diketone produced by *P. mariae-lebouriae* was lower than that produced by *P. minimum*, the influence of irradiance on the temporal pattern of the β-diketone production was similar (Fig. 1). In *P. mariae-lebouriae*, reduction of the irradiance from 160 to 120 $\mu Ei\cdot m^{-2}\cdot s^{-1}$ had no significant influence on the amount of β-diketone produced. An increase in irradiance to 270 or a decrease to ~90 $\mu Ei\cdot m^{-2}\cdot s^{-1}$ inhibited β-diketone production.

TABLE 1. Species examined for the production of the β-diketone. Final cell yield and the maximum recorded β-diketone concentration (±1 SE, $N = 3$) are presented for cells at 160 $\mu Ei\cdot m^{-2}\cdot s^{-1}$ and 18°C, except for *P. mariae-lebouriae*, which was grown at 22–23°C. All cultures were grown in P-deficient batch cultures. Extracellular concentration of the β-diketone was monitored daily. North East Pacific Culture Collection number is given in parentheses for the appropriate species. ND = not detectable.

Species	Final cell yield (10^7 $cells\cdot L^{-1}$)	Maximum β-diketone ($\mu g\cdot L^{-1}$)
P. minimum		
(Pavillard) Schiller (No. 96)	3.3	39.6±7.2
P. minimum var. balticum		
(Pavillard) Schiller (No. 115)	0.7	ND
P. micans		
Ehrenb. (No. 104)	2.1	10.3±2.7
P. gracile		
Schuett (No. 104)	3.0	ND
P. maximum		
(Gouret) Schiller (No. 250)	1.7	ND
P. mariae-lebouriae		
(Parke et Ballentine) Hulburt	3.5	10.1±3.4

At each irradiance where β-diketone was detectable, the temporal pattern of production was the same regardless of the absolute amount of material produced. At the onset of phosphate starvation (day 11 for *P. mariae-lebouriae*), the β-diketone was produced rapidly and maintained at a constant level in the medium. Similar patterns of production were seen under all other experimental conditions.

The production of the β-diketone under a light–dark cycle (18 h light : 6 h dark) suggests that the production of the β-diketone is light controlled (Fig. 2). While only one experiment was performed, there was no evidence of β-diketone production during the dark period. At the onset of the light, there was rapid production during the first few hours, shifting to a gradually slower rate of release. The rapid release of the β-diketone may be due to the conversion of an intracellular precursor pool that forms during the dark period. While the kinetics of production cannot be fully appreciated with a single experiment, the link of production with the onset of the light period suggests a photocontrol mechanism.

Salinity had a significant influence on the final extracellular β-diketone concentration. *Prorocentrum minimum* had maximum growth rates over a wide range of salinities (from 15 to 30‰) (Fig. 3A). Maximum β-diketone production had a restricted salinity range of 20–30‰. At salinities less than 20‰, the amount of extracellular β-diketone produced decreased rapidly. *Prorocentrum mariae-lebouriae* had a very different salinity tolerance range. Salinities of 10–15‰ were required to give maximum growth rates (Fig. 3B). Full-strength medium (27‰) reduced the growth rate by more than 50% and nearly eliminated the β-diketone production. Growth rates and β-diketone production were also reduced at salinities less than 10‰. Maximum extracellular β-diketone concentrations were achieved at salinities below those providing maximum growth rates.

Temperature also had a profound effect on extracellular β-diketone production (Fig. 4). Temperatures less than 15°C reduced the amount of β-diketone produced from 50 to less than 3 $\mu g\cdot L^{-1}$. There was no detectable β-diketone when cells were grown at 5°C.

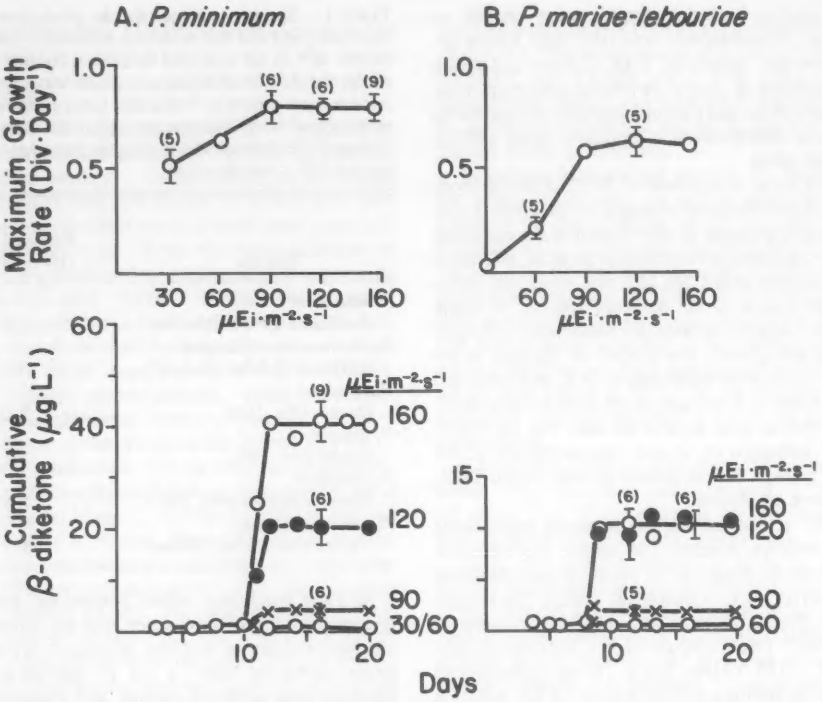

FIG. 1. Influence of irradiance on the maximum growth rate (upper panel) and the temporal production of the extracellular β-diketone (lower panel) for (A) *P. minimum* and (B) *P. mariae-lebouriae*. Batch cultures eventually became phosphate deficient. Replicate values are mean ± 1 SE (*N* in parentheses).

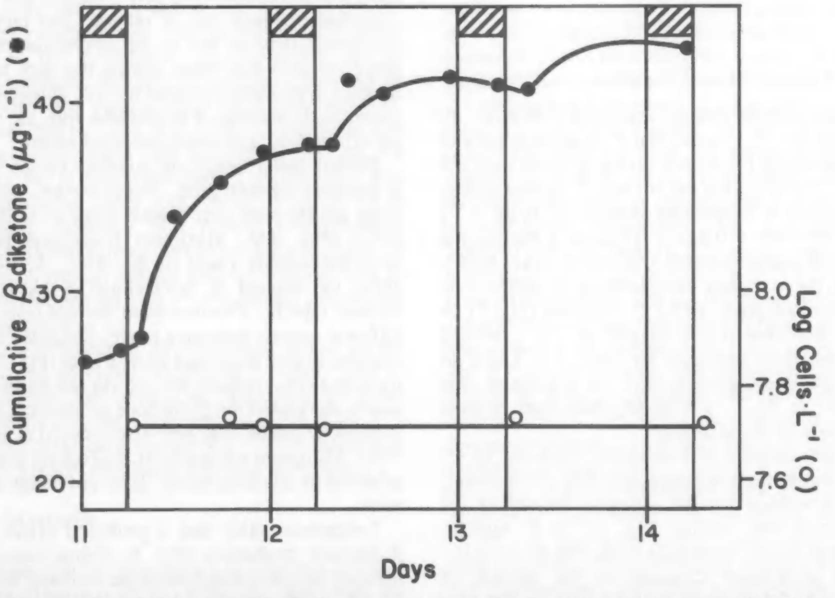

FIG. 2. Cumulative extracellular β-diketone concentration for *P. minimum* grown under a 18 h light: 6 h dark regime. Only the senescent phase of the batch culture is shown.

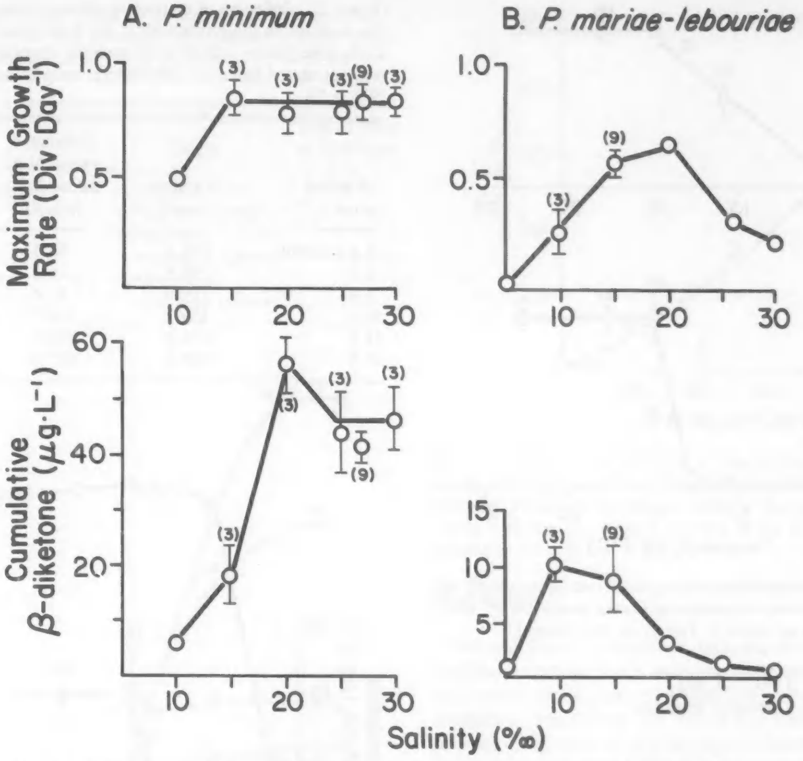

FIG. 3. Influence of salinity on the growth rate (upper panel) and the maximum β-diketone concentration (lower panel) for (A) *P. minimum* and (B) *P. mariae-lebouriae*. Replicate values are means ± 1 SE (*N* in parentheses).

Production under Different N:P Ratios

At very low N:P ratios (N:P = 2) a large amount of inorganic precipitate formed (C = 2.8%, N = 1.4%, N = 0.3%, P = 6.9%). Because autoclaved medium without cells (N:P = 2) failed to show any precipitate, formation of this precipitate was either stimulated by the presence of the cells or the activity of the cells. The β-diketone was not produced at this ratio.

At N:P ratios from 5 to 22 (N-deficient growth), the level of β-diketone produced was constant at 20 $\mu g \cdot L^{-1}$ (Fig. 5). As the cells shifted to P-deficient growth conditions (N:P = 27–35), production doubled to about 40 $\mu g \cdot L^{-1}$. Phosphate-deficient cells exposed to increasing concentrations of ambient nitrate produced decreasing amounts of β-diketone. At N:P ratios greater than 60, no β-diketone was produced.

To test whether the reduction in β-diketone production at N:P = 40 was a function of the actual ratio or the amount of excess nitrate in the culture medium, a series of phosphate-deficient culture experiments were performed in which the N:P atomic ratio was kept constant at 40 but the amount of phosphate added was increased or decreased. As the amount of excess nitrate in the culture medium decreased, the amount of β-diketone normalized on a per cell basis increased, reaching values similar to the maximum amount of the β-diketone produced under P limitation (Table 2). Conversely, an increase in the amount of excess nitrate, at a constant N:P ratio of 40, completely inhibited the β-diketone production.

A similar experiment to examine the influence of excess phosphate at N:P = 2 failed to show an increase in the β-diketone production. In all experiments, a large amount of precipitate formed that may have interfered with cellular processes and reduced the amount of excess phosphate, because the precipitate contained large amounts of phosphate. The amount of precipitate could not be quantified, because much of the material adhered tenaciously to the glass flask.

The methanol extracts of *P. minimum* cells grown at N:P = 5 were examined by TLC to determine if the difference in extracellular β-diketone concentration was due to intracellular storage when cells were nitrate limited. Cells from N:P = 5 and N:P = 27 were extracted with methanol and run with a β-diketone standard in a $CHCl_3$–ethyl acetate (1:1) TLC system. Neither of the cell extracts contained the β-diketone, although there were obvious differences in the cellular profiles. Cells grown at N:P = 5 contained $FeCl_3$ positive material with R_f values greater than the β-diketone (0.6 and 0.89 vs. 0.37 for the β-diketone). Neither of the $FeCl_3$ positive spots gave the characteristic red–brown color of the β-diketone.

Production under Iron-Deficient Growth Conditions

Prorocentrum minimum cells grown under iron-limited culture conditions had a pattern of extracellular β-diketone production similar to the pattern from phosphate-deficient cultures, but the absolute amount of the β-diketone produced was substantially lower (Fig. 6). Only cells grown in the ESNW–Fe medium produced the β-diketone.

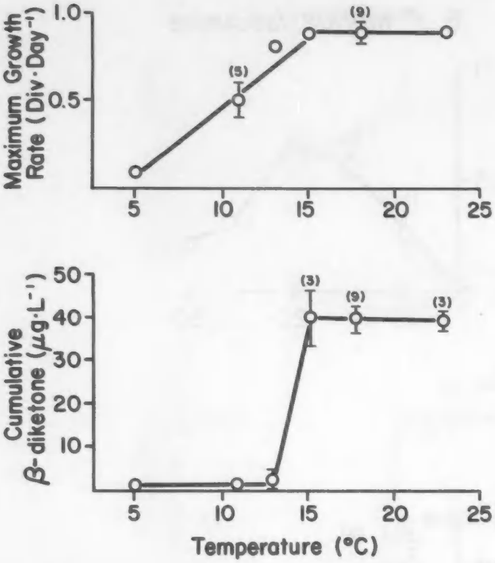

FIG. 4. Influence of temperature on the growth rate (upper panel) and the maximum β-diketonea concentration (lower panel) for *P. minimum*. Replicate values are mean ± 1 SE (*N* in parentheses).

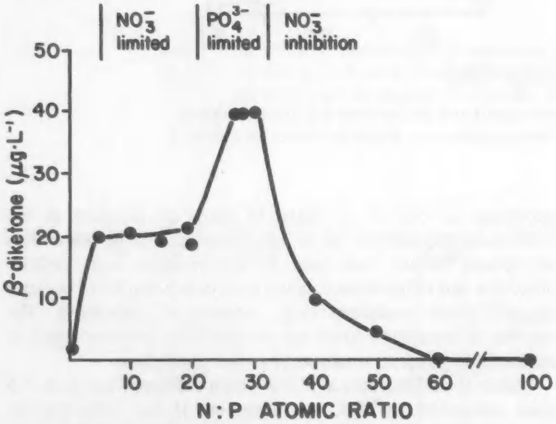

FIG. 5. Influence of the initial N:P atomic ratio in the medium on the maximum β-diketone concentration for *P. minimum*. Final cell yields for all experiments were equivalent.

The addition of NH_4VO_3 had no effect on the growth parameters measured (growth rate and final cell yield were equivalent to the iron-sufficient controls), but it completely inhibited the production of the β-diketone (Table 3). The addition of iron to the treated medium reestablished maximum extracellular concentrations.

Bacterial Bioassays

All bacteria formed smooth, uniform lawns within 48 h of inoculation. One marine isolate (*Pseudomonas acidivaris*) was insensitive to β-diketone (Table 4). *Chromobacterium* sp. was the most sensitive based on the size of the zone of inhibition.

Since the disc bioassay is not a reliable predictor of the effective concentration of inhibition, *Chromatobacterium* sp. was grown in liquid culture with β-diketone concentrations ranging from 0 to 100 µg·L⁻¹. At β-diketone concentrations greater than 25 µg·L⁻¹, the final cell yield was reduced. Initial rate of growth was not affected (Fig. 7). Complete suppression of growth was not recorded in this range of β-diketone concentrations. Growth was monitored for 14 d to ensure that the bacteria could not adapt to the β-diketone concentration. For a 50% inhibition of the short-term heterotrophic uptake of

TABLE 2. Influence of increasing ambient nitrate concentration in the medium on the production of the β-diketone by *P. minimum*. Cells were grown in N:P = 40 medium. Excess N in the medium was calculated based on a theoretical nutrient equilibrium ratio of N:P = 25.

P added (µmol·L⁻¹)	N added (µmol)	Calculated excess N in the medium (µmol)	Extracellular β-diketone (µg·10⁷ cells⁻¹)
21.9 (control)	591.3	43.8	6.3
2.7	109.8	36.1	5.8
5.4	219.6	82.4	3.7
10.9	439.2	164.7	0.9
21.9	878.4	330.9	0.8
43.9	1756.8	657.8	0.0

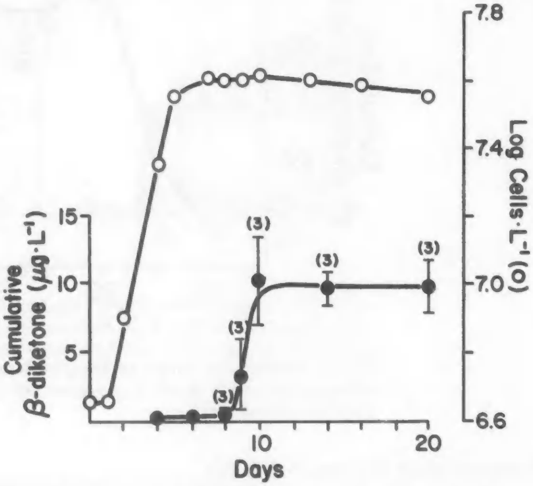

FIG. 6. Changes in the β-diketone concentration (●) and cell numbers (○) during exponential growth and during senescence in iron-starved batch cultures of *P. minimum*. Replicate values are mean ± 1 SE (*N* in parentheses).

TABLE 3. Influence of the addition of vanadium on the β-diketone production in the iron-starved batch cultures of *P. minimum*. Cultures were harvested at day 15 of batch culture growth.

Growth conditions	Maximum β-diketone produced (µg·L⁻¹)
Iron limited	10.9
Iron limited with a 4 × 10⁻⁸ mol NH_4VO_3·L⁻¹ added	0.0
Iron limited with a 8 × 10⁻⁸ mol NH_4VO_3·L⁻¹ added	0.0
Iron sufficient (control)	38.3

TABLE 4. Description and origin of the bacterial species examined. Qualitative results of the disc bioassay are presented in terms of the size of the zone of inhibition (0, = no inhibition; +, <0.5 cm; ++, >0.5 cm).

Species	Origin	Size of zone of inhibition
Pseudomonas acidivaris	ATCC No. 9355	0
Vibrio sp.	ATCC No. 14400	+
Flavobacter sp.	*Scrippsiella sweeneya* culture	++
Chromobacterium sp.	*Scrippsiella sweeneya* culture	++

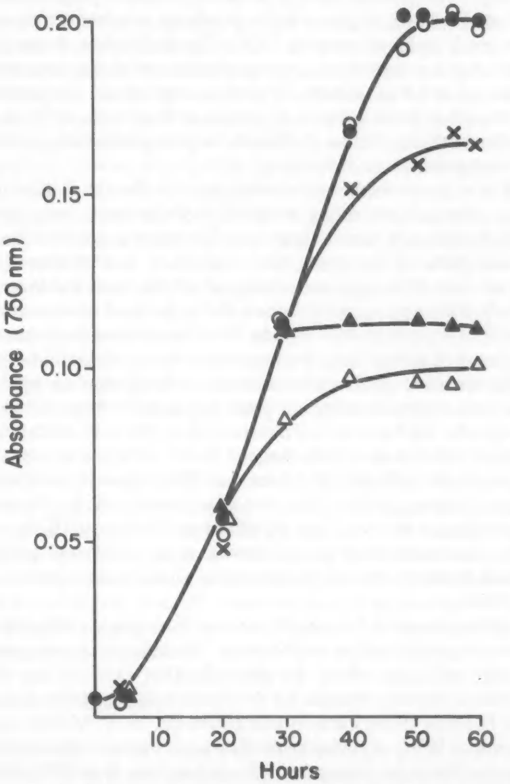

FIG. 7. Influence of the addition of the β-diketone on growth of *Chromobacterium* sp. Growth was measured by changes in absorbance at 750 nm. Cells were grown at room temperature in ISOL medium with varying concentrations of the β-diketone. ○, control; ●, 10 μg·L^{-1}; ×, 25 μg·L^{-1}; ▲, 40 μg·L^{-1}; △, 75 μg·L^{-1}.

natural populations, 100 μg β-diketone·L^{-1} was required (Fig. 8). However, concentrations as low as 20 μg·L^{-1} inhibited the heterotrophic uptake (10% reduction), and at β-diketone concentrations (50 μg·L^{-1}) typically produced by laboratory cultures of *P. minimum*, heterotrophic uptake was inhibited by about 15%. The β-diketone concentrations from laboratory cultures would overestimate the actual concentration of extracellular β-diketone produced because of the large discrepancies in cell concentrations.

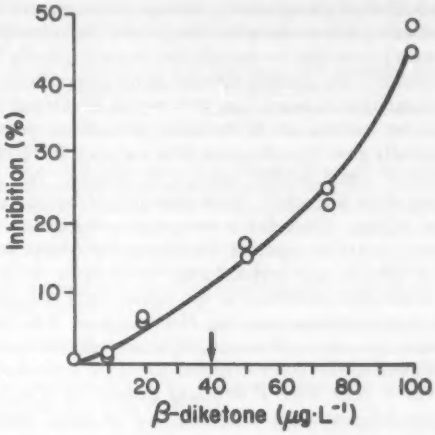

FIG. 8. Effect of the addition of the β-diketone on heterotrophic uptake of [^{14}C]glucose by a natural population. Bacteria were collected from the Fraser River plume, Strait of Georgia, Vancouver, B.C. Arrow indicates the relative amount of the β-diketone produced by laboratory cultures.

Discussion

Several lines of evidence indicate that the control of the β-diketone production is under the same control processes as the total extracellular carbon release (composed mostly of primary organics). Production of the β-diketone is related to the physiological state of the cell and is limited to the stationary phase of growth. This is the case for many extracellular organics (Guillard and Wangersky 1958; Marker 1965; Bolze and Soeder 1978; Melkonian 1979). However, in the case of the β-diketone produced by *P. minimum*, the production in the stationary phase is tightly controlled. Release of the β-diketone is not gradual, suggesting a "leaking" of cellular contents, but rather, there is a short period of rapid production followed by an equally rapid cessation of β-diketone production after a short (1–2 d) period of time. The restricted period of production suggests the exhaustion of cellular precursors available for β-diketone production.

Irradiance was a strong influence in controlling the β-diketone production. Increased irradiances favor the release of dissolved organic carbon (DOC) by marine phytoplankton (Fogg et al. 1965; Hellebust 1965; Nalewajko 1966) and the same can be seen for the production of the β-diketone. In the latter case, the extent of the production increased nonlinearly over the range of irradiances. Similar light control of DOC from *Skeletonema* has also been shown (Ignatiades and Fogg 1973). The production of β-diketone did not occur if light was limiting to growth. This is not necessarily true if one examines DOC release. Kuenzler (1970) found DOC release to be constant over a wide range of irradiances, including light levels limiting to growth rates. The amount of the β-diketone production was not controlled extensively by temperature, although production of the β-diketone ceased when temperatures reduced the growth rate of *P. minimum*.

The influence of nutrient deficiency on the production of the extracellular β-diketone suggests a strong control by the physiological state of the cell. Shifting from N-deficient to P-deficient growth conditions shifts the carbon metabolism of the cell. Enzymes (such as polyphosphokinase, polyphosphatase, and alkaline phosphatase), storage products, and energy intermediates (such as adenylate charge and adenosine triphosphate) are all controlled by the deficient nutrient (Healey 1975). The influence of the limiting nutrient on the composition of the extracellular exudate is less well understood. Myklestad (1977) clarified the control of N:P ratios on cellular processes. Exponentially growing cells show little variation in the cellular composition, since neither nutrient is limiting, but as the stationary phase is reached, the composition of the medium has profound effects. The cellular composition changes to reflect the composition of the medium. Examining the influence of N:P ratios on growth and carbohydrate metabolism, Myklestad (1977) noted little difference in the extracellular polysaccharides at concentrations over the N:P range of 0.4–100 for *Skeletonema costatum*. However, Myklestad did find increased levels of extracellular polysaccharide during the senescent stage of growth if cells were P deficient instead of N deficient. Phosphate-deficient cells produced two to three times the amount of extracellular polysaccharide compared with nitrate-deficient cells. A similar trend was shown for extracellular polysaccharide production in stationary *Chaetoceros affinis* cultures (Myklestad 1977).

The influence of the N:P ratio on the extracellular production of the β-diketone is similar in several significant ways. When *P. minimum* was N deficient, a constant amount of β-diketone was produced at 20 $\mu g \cdot L^{-1}$. When cells shifted to P deficiency there was an increased amount of the β-diketone produced (40 $\mu g \cdot L^{-1}$). But at N:P ratios greater than 30, it was the amount of excess nitrate that inhibited the amount of the β-diketone produced. The control of extracellular metabolites by excess nutrients has been shown for many bacterial and fungal metabolites (Weinberg 1970; Drew and Demain 1977; Martin and Demain 1980).

The reduced amount of the β-diketone produced under iron-deficient growth conditions compared with P-deficient cultures suggests that the β-diketone was not directly linked to the high-affinity iron transport system, even though the β-diketone forms an iron complex and has the potential to chelate metals (Andersen et al. 1980).

The addition of vanadium to iron-limited cultures of *P. minimum* was a critical experiment. Vanadium is a trace metal found commonly as a constituent of marine algae (O'Kelly 1974), but it is not necessarily an essential nutrient. The addition of vanadium to the culture medium inhibited the formation of δ-aminolevulinic acid, which is a key step in the regulation of chlorophyll production (Meisch and Bielig 1975). This is the same point at which iron limitation affects the cell's physiology (Jones 1975). A second influence of vanadium on the cell's physiology was the stimulation of the photoinduction of carotenogenesis (Shropshire 1980). The addition of vanadium to *P. minimum* inhibited the β-diketone production rather than stimulated the norcarotenoid release, providing further evidence against the β-diketone as a nonselective photooxygenation product of carotenoids.

It is apparent from the limiting nutrient experiments that the processes controlling the β-diketone production are regulated by two nutrient-related criteria: (1) the type of limiting nutrient (P, N, or Fe) and (2) the amount of excess nitrate available in the culture medium.

Several points can be stressed. The production of the extracellular β-diketone is a normal physiological process related to the carbon flow within the cell. The β-diketone is formed immediately prior to release and is never found intracellularly. The production is a function of the nutrient environment of the cells and is mediated by photochemical control of compound formation.

Relating the production of the β-diketone to the ecology of *P. minimum* is difficult. Unlike prorocentrin (the siderophore produced by *P. minimum* (Trick et al. 1983)), the role of the β-diketone cannot be directly related to the physiological response of the cell.

The link between the production of extracellular β-diketone and the formation of *P. minimum* blooms is especially open to speculation. Researchers examining natural phytoplankton blooms have noticed that even though cells are severely stressed or senescent, there is no resulting increase in the amount of extracellular carbon release (Herbland and Dandonneau 1975; Mague et al. 1980). Although the quantity of extracellular organics may not change significantly, the quality might change. The β-diketone comprises only a small proportion of the total released organics and is produced at levels that would not result in an increase in DOC. The β-diketone, however, does shown antibacterial activity when tested at the concentrations recorded in culture. If the amount of the β-diketone produced in batch cultures is similar to that in the field, it is predicted that sufficient β-diketone will be produced to inhibit natural populations of bacteria.

Part of the problem in evaluating the role that the β-diketone may play in controlling bacterial populations is our poor understanding of the link between phytoplankton and bacteria. Researchers in the past have suggested that antibacterial metabolites from algae are not important (Carlucci and Pramer 1960), but recent work by Aubert and co-workers (Aubert et al. 1968, 1970; Aubert and Pesando 1971) has provided substantial evidence of strong algae–bacteria interactions. Metabolites that influence algae–bacteria interactions can be nonspecific inhibitors such as tannins or organic acids (Lucas 1955; Nigrelli 1958) or specific, uncharacterized metabolites produced by laboratory cultures (Bellet al. 1974; Kogure et al. 1979) or by natural populations (Sieburth 1971; Moebus 1972). Characterized antibacterial agents produced by phytoplankton include acrylic acid from *Phaeocystis pouchetii* (Guillard and Hellebust 1971) and *Phaeodactylum tricornutum* (Brown et al. 1977) and goniodomin from the marine dinoflagellate *Goniodoma* (Sharma et al. 1968).

There are several ways of viewing the organic carbon link between phytoplankton and bacteria. The link can be a one-way carbon exchange where the phytoplankton exudates are the source of organic material for the bacteria (Smith 1974; Smith and Higgins 1978; Larsson and Hagström 1979; Meffert and Overbeck 1979), and the extent of carbon transfer to the bacteria can be substantial. Estimates indicate that from 25 to 50% of the total fixed carbon is incorporated into the bacteria (Andrews and Williams 1971; Hagström et al. 1979; Larsson and Hagström 1979). These estimates suggest that a large part of the bacterial carbon requirement is met by phytoplankton exudates. To strengthen the link between phytoplankton and bacteria, Larsson and Hagström (1982) have reintroduced the concept of the "phycosphere." The phycosphere, as it was originally described by Bell and Mitchell (1972), is a zone surrounding each phytoplankton cell composed of a high concentration of algal exudates. The zone contains all extracellular organics including bacterial attractants (Kogure et al. 1982) and antibacterial agents. Thus, the epiphytic bacterial population of a phytoplankton cell would be a function of the control of extracellular organic production.

This relationship has been poorly characterized. Bacteria gain by having a high concentration of readily available organics and a constant source of material. The phytoplankton may gain required organics such as vitamins or growth factors from the bacteria, but almost no evidence exists for the transfer of organics from bacteria to phytoplankton (Haines and Guillard 1974).

Exponentially growing cells would develop a metabolically active bacterial population with the phycosphere, especially if the bacteria show a chemotactic response to algal exudates (Bell and Mitchell 1972; Sjoblad and Mitchell 1979). As phytoplankton become nutrient deficient, the amount of organics released also decreases. The bacteria become organic stressed and may initiate the degradation and remineralization of the associated phytoplankton by the production of extracellular enzymes. In this situation the bacteria could produce sufficient concentrations of extracellular enzymes to degrade the host.

A phytoplankton cell that forms a resting stage may alter its structure to resist bacterial attack. However, species such as *P. minimum*, for which no alternate stage is known, could produce antibacterial metabolites such as the β-diketone to reduce the degradative activity of the associated bacterial population.

The pattern of production of the β-diketone lends itself to this hypothesis. Prior to the β-diketone production, bacteria associated with *P. minimum* would have a favorable environment for heterotrophic activity. With the heterotrophic mechanism intact, the β-diketone is produced in a single rapid pulse, creating a toxic phycosphere. Associated bacteria are inhibited prior to excretion of extracellular degradative enzymes. The activity of the associated bacteria is influenced by the chemical composition of the substances released and the pattern of release. Strict control of production is necessary to ensure that the β-diketone is released in sufficient quantities to eliminate the associated bacterial activity under physiological conditions that are temporally severe.

The study of the role of heterotrophic bacteria and microflagellates in degrading and remineralizing the organics in marine waters is of primary importance in the understanding of microbial interrelationships (Strickland 1971). To understand the phytoplankton–bacteria relationships both the quantity and the quality of organics must be considered. The production of the norcarotenoid from *P. minimum* may be an example of the severing of organic links between trophic levels by extracellular phytoplankton exudates.

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Growth and Survival of Developing Steelhead Trout (*Salmo gairdneri*) Continuously or Intermittently Exposed to Copper[1]

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Seim, W. K., L. R. Curtis, S. W. Glenn, and G. A. Chapman. Growth and survival of developing steelhead trout (*Salmo gairdneri*) continuously or intermittently exposed to copper. Can. J. Fish. Aquat. Sci. 41: 433–438.

Steelhead trout (*Salmo gairdneri*) were continuously or intermittently exposed to copper over a 78-d period from 6 d postfertilization through 5 wk postswimup. Copper was introduced into the intermittent exposure chambers for 4.5 h each day. Continuously exposed fish survived and grew better than intermittently exposed fish at the same daily mean copper concentrations. Intermittently exposed fish accumulated significantly more copper than those continuously exposed at the same daily mean concentrations. Results demonstrated that data derived from continuous laboratory exposures should not be directly converted to water quality criteria where toxicant concentrations in natural waters are temporally variable.

Des truites steelhead (*Salmo gairdneri*) ont été exposées de façon continue ou intermittente à du cuivre pendant une période de 78 jours, à partir du 6^e^ jour après la fécondation jusqu'à 5 sem inclusivement après le moment de nage vers le haut. Dans l'exposition intermittente, le cuivre a été introduit dans les chambres pendant 4,5 h quotidiennement. Les poissons soumis à une exposition continue ont eu une meilleure survie et croissance que ceux soumis à une exposition intermittente aux mêmes concentrations moyennes quotidiennes de cuivre. Les truites exposées de façon intermittente ont accumulé une quantité de cuivre nettement supérieure à celle des poissons exposés continuellement aux mêmes concentrations moyennes quotidiennes. Ces résultats indiquent que les données dérivées d'exposition continues en laboratoire ne devraient pas être converties directement en critères de qualité de l'eau dans les cas où la concentration de toxique dans les eaux naturelles varie dans le temps.

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Cyclic industrial processes, variable stream flow, chemical use patterns such as cooling tower chlorination or aerial pesticide application, contaminated precipitation, and surface water runoff contribute to the occurrence of transient elevations of pollutant concentrations in natural waters. While exposure of aquatic organisms to toxicants in receiving waters is often variable both in terms of duration and concentration, most data describing effects of toxicants on aquatic organisms represent conditions of continuous exposure to essentially constant concentrations.

Acute (48 or 96 h) effects of fluctuating toxicant concentrations on trout survival have received some attention. Brown et al. (1969) reported reduced survival time when ammonia concentrations fluctuated between 1.5 and 0.5 times 96-h LC50 at 2 h but not at 1 h intervals. No difference was noted in survival of fish exposed either to fluctuating or constant concentrations of zinc or mixtures of zinc and ammonia, however. Brown's results for 2-h fluctuation of ammonia were consistent with those of Thurston et al. (1981), which demonstrated that 96-h LC50's based on mean values of fluctuating ammonia concentrations were lower than LC50's for fish exposed continuously. This phenomenon was not demonstrated for exposures extended to 14 and 28 d when 6 h on, 6 h off and 12 h on, 12 h off regimens were tested.

Ingersol and Winner (1982) chronically exposed *Daphnia* to cadmium and copper at the respective 48-h LC50 for sufficiently long daily periods to equal the "no-observable-effect" mean concentration for constant exposure. This was an exposure period of 70 min for cadmium and 360 min for copper. Copper reduced survival and brood size of *Daphnia*, while no effect was attributed to cadmium exposure. A slower uptake of cadmium as compared with copper was suggested to explain the difference in results.

The above data indicated that criteria or standards developed from continuous exposure data may or may not be appropriate for the protection of aquatic organisms in intermittent exposure situations. The 24-h mean toxicant concentration and the maximum, or peak, toxicant concentration have both been proposed for protecting aquatic organisms (Anonymous 1980). Our objective in this study was to examine the chronic relationships between continuous and intermittent exposures and the survival, growth, and development of salmonid embryos and larvae. We also evaluated the proposed 24-h mean and maximum concentration criteria through their application to results of these laboratory experiments. The approach was to compare the effects of continuous exposures with the effects of intermittent exposures to single daily toxicant peaks of about a

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7-h duration. Accumulation of the toxicant was measured to provide a basis for understanding differences observed. Copper was selected for our experiments because its toxicity has been extensively studied, measurement was relatively simple, and it remains a widespread environmental contaminant.

Methods

Steelhead trout (*Salmo gairdneri*) eggs and sperm were obtained from the Alsea Fish Hatchery (Oregon Department of Fish and Wildlife). Eggs taken from three females were pooled and fertilized with sperm from four males. The eggs were allowed to water harden for 1 h prior to transport (in water) to the laboratory. Upon arrival, 60-mL aliquots (approximately 240 eggs) were placed as a single layer in each of 26 incubation chambers.

Experimental Apparatus

A flow-through dilution apparatus was constructed by a modification of the design of Chadwick et al. (1972). Well water flowed through plastic float valves into two separate headboxes, where the temperature was maintained at 12°C by thermostatically controlled, stainless steel immersion heaters. Room temperature was also held at about 12°C. Flow rates of dilution water and stock toxicant solution (3 mg Cu/L) were measured daily and necessary adjustments were made. The total flow to each incubation chamber was approximately 400 mL/min. The stock solution was continuously circulated between the stock supply and the delivery manifold; this mixing, along with addition of 0.25 mL of concentrated nitric acid per litre of stock copper chloride solution, maintained a uniform stock solution. A timer-controlled pump regulated inflow of stock solution to the delivery manifold, which served intermittent exposure chambers. The timer was set to introduce stock solution into the test system from 10:00 to 14:30 daily.

Incubation chambers used in the first 7 wk of the study were constructed from two-piece, polypropylene Buchner funnels firmly seated in wooden cross-supports. The upper compartment that housed the embryos was an 18-cm-diameter, 15-cm-high cylinder with a perforated bottom and open top. The lower compartment's spout was fitted with polyethylene (Tygon®) tubing to supply water from the delivery manifold. Water flowed up through the test exposure chamber and was discharged through a number of small holes drilled just below the top. Each incubator was covered with a plate of untreated wood to exclude light. The 50% replacement time in the incubators was about 10 min (Sprague 1969).

The final 5 wk of the study (beginning when the "swimup" stage was reached in control fish) were conducted in 60-L aquaria equipped with outlet tubes to maintain a constant volume of 30 L. These tanks were coupled to the delivery manifold with PVC tubing. The total flow to each aquarium was approximately 800 mL/min, and the 50% replacement time was about 25 min (Sprague 1969).

Procedures

Water chemistry samples were removed weekly from a control chamber and a chamber at the highest remaining copper exposure concentration. Dissolved oxygen remained near air saturation throughout the study (range 9.3–10.0 mg/L). Hardness averaged 120 mg/L and alkalinity averaged 126 mg/L as calcium carbonate. Neither parameter deviated from the mean by more than 4 mg/L over the study. The pH of control water varied from 7.4 to 7.9 over the 78-d study. The presence of nitric acid in the stock copper solution decreased pH of exposure water by less than 0.1 pH unit at copper concentrations below 300 μg/L and by 0.3 pH unit at 465 μg Cu/L. Copper concentrations in water were measured weekly. Water samples from continuous exposure tests and those taken near the end of the 4.5-h period of copper dosing were acidified to 0.01 mol nitric acid/L and analyzed for copper by atomic absorption spectrometry utilizing a graphite furnace (Perkins-Elmer model HGA 2000). Several duplicate water samples were filtered through 0.45-μm filters prior to acidification; at the pH of exposure water, over 95% of the copper was soluble at 400 μg/L but only about 60% was soluble at 4500 μg/L.

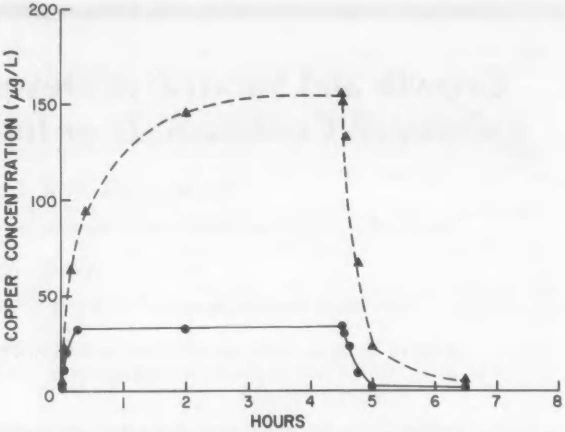

FIG. 1. Typical time course of total copper concentration for a high and a low concentration peak. Toxicant was introduced daily for a single 4.5-h period.

Frequent measurement of copper concentration during several 4.5-h dosing periods indicated that peak concentrations were reached within about 2 h and levels above background persisted for about 7 h (Fig. 1). Areas under the time–concentration curves for each intermittent exposure concentration were determined by planimetry and related to the peak concentration. These data were used to calculate 24-h mean concentrations for intermittent exposures based on the weekly samples of peak concentrations. The copper concentration in control water averaged 3 μg/L.

Copper exposure of steelhead embryos in the incubation chambers was begun 6 d following fertilization. Five days later, visibly discernible dead embryos and unfertilized eggs were removed and counted. Two weeks later, daily tabulation of hatching and of mortality began, and daily removal of dead embryos or alevins was initiated. At 7 wk postfertilization, when all control fish had hatched and reached swimup stage, subsamples of 100 alevins (fewer for the highest intermittent and continuous concentrations, which suffered high mortality) were transferred from the incubation chambers to aquaria and the same exposure patterns were continued. These fish were fed at 08:00 each day with a ration of Oregon Test Diet (Sinnhuber et al. 1977) at 12% of body weight. The ration supplied to each tank was adjusted weekly for mortality and loss to sampling; rations were also adjusted for fish wet weights determined every 2 wk on a subsample of fish that were weighed in water and then returned to the aquarium. No attempt was made to quantify the food not consumed, but the aquaria were cleaned daily to remove uneaten food.

TABLE 1. Time course for responses of developing steelhead trout to continuous and intermittent copper exposures. Data represent percent survival and mean dry weight (parentheses).[a]

	Concentration (µg/L)		Days postfertilization										
	Mean	Peak	11	22	31[b]	38	45	49[c]	56	63	70	78	85
Continuous exposure	3	(control)	100	97.5	97.3 (3.2±0.2)	96.3	95.9 (18.9±1.6)	95.9	94.5	92.6 (69.1±3.3)	92.6	92.6	92.6 (134.2±10.3)
	6		100	99.4	98.6 (3.7±0.1)	98.4	98.4 (18.5±0.4)	97.5	97.5	97.5 (73.3±3.7)	97.5	97.5	97.5 (141.0±8.4)
	9		99.5	98.4	98.1 (3.8±0.2)	98.1	96.1 (19.9±1.2)	95.8	95.8	95.8 (67.4±3.7)	95.8	95.8	95.8 (151.6±7.7)
	16		99.8	99.0	97.4 (4.1±0.1)	96.5	95.9 (18.6±1.4)	93.9	93.0	93.0 (72.7±3.3)	93.0	93.0	93.0 (153.3±9.0)
	31		99.6	98.9	97.6 (3.2±0.2)	96.6	95.9 (16.0±0.9)	94.1	94.1	91.3 (50.9±2.2)*	89.4	89.4	89.4 (107.5±6.9)*
	57		99.8	99.2	98.6 (2.8±0.2)	96.4	92.2 (12.1±1.2)*	89.9	81.2	78.3 (33.9±2.9)*	73.5	73.5	73.5 (47.2±3.9)*
	121		88.4	35.6	23.8 (2.5±0.2)*	19.7	16.3 (9.4±3.5)*	15.3	8.6	6.6 (15.8±0.3)*	0		
Intermittent exposure	6	21	100	99.8	99.3 (3.0±0.2)*	98.8	98.0 (18.7±1.0)*	96.0	96.0	93.8 (62.3±3.0)	93.8	93.8	93.8 (139.1±8.4)
	8	35	100	99.6	98.8 (3.1±0.2)	98.2	97.6 (15.5±1.3)	97.4	96.4	96.4 (62.3±3.0)	96.4	96.4	96.4 (131.8±10.4)
	16	76	99.7	99.7	99.2 (2.9±0.3)	98.6	98.3 (15.8±0.7)	96.5	96.5	96.5 (50.5±2.9)	96.5	96.5	96.5 (97.4±5.4)*
	30	149	99.8	99.6	98.6 (2.5±0.2)	96.3	95.6 (15.6±0.8)	93.2	93.2	93.2 (46.8±2.9)*	93.2	93.2	90.2 (56.9±3.6)*
	51	263	99.8	99.2	81.6 (2.5±0.2)*	67.8	65.5 (11.4±1.1)*	64.5	55.7	46.2 (29.5±2.4)*	40.1	36.0	31.7 (36.1±2.5)*
	109	465	85.1	50.0	29.9 (2.0±0.3)*	4.2	3.4 (6.5±1.1)*	3.4	0				

[a] Mean ± SE (mg mean dry weight) for 10 or 20 fish (*significantly different from control at $P = 0.05$).

[b] Hatching preceded this sample by 2 or 3 d.

[c] Feeding of fish began the day following this sample.

Fish from all exposure treatments were periodically sampled for determination of dry weight. A subsample of 10–20 fish from each group was killed in MS-222 solution, any remaining yolk was dissected off, and individual animals were placed in preweighed aluminum pans. Following drying for 96 h at 70°C the residual material was cooled in a desiccator and weighed on an analytical balance. The first sample was taken when 50% of the eggs had hatched, the second 2 wk later, the third 2 wk after feeding began, and the fourth 3 wk later (27–29, 41, 63, and 84 d postfertilization, respectively).

Following 8 and 11 wk of exposure, individual fish were dried, weighed, and then digested at 100°C in 1 mL of concentrated nitric acid. Digests were resuspended in 0.1% nitric acid and analyzed on a Perkins-Elmer model 305B atomic absorption spectrometer equipped with a graphite furnace. Similar analysis of bovine liver from the U.S. Bureau of Standards measured the efficiency of copper recovery.

Since live fish were removed during the course of the experiment for destructive analysis, survival could not be directly calculated as the quotient of number living at the end of the test and initial population size. Rather, such calculations were made for each sampling period and the overall mortality was calculated as follows:

$$q = 1 - (l_{x_1} \cdot l_{x_2} \dots \cdot l_{x_n})$$

where q is the estimated proportion dying and each l_x is the survivorship proportion during the respective sampling period.

Acute Mortality

The 96-h LC50 was determined for a cohort of control fish 1 wk following termination of the embryo–larval test. The fish were held in 30-L aquaria with a flow rate of 400 mL/min with continuous concentrations of 13, 29, 42, 73, 99, and 171 µg Cu/L and, for intermittently exposed fish, peak concentrations of 138, 200, 330, and 490 µg Cu/L.

Statistics

Analysis of variance followed the methods of Williams (1971). Curve fitting techniques applied were described by Netter and Wasserman (1974). EC50 estimates were calculated by the methods of Snedecor and Cochran (1976). LC50 estimates were based on the logit model.

Results

Survival

Details on the time courses for responses of the organisms to copper exposures are provided in Table 1. Survival of steelhead embryos and alevins exposed continuously to copper concentrations from 3 to 30 µg/L was 90% or greater. Survival was about 74% at 57 µg/L, with most of the mortality occurring after the fish began to feed. Steelhead continuously exposed to copper at 121 µg/L had all died by the 9th wk of the experiment, with only about 25% surviving past the embryonic stage. Embryos and

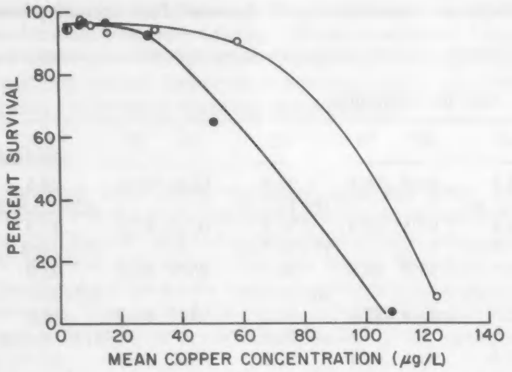

FIG. 2. Comparison of percent survival of steelhead following 78 d of intermittent or continuous exposure. Concentrations are expressed in terms of the mean copper concentrations measured in exposure water throughout the experiment. ○, continuous exposure; ●, intermittent exposure; ◐, control.

alevins exposed intermittently to copper at peak concentrations up to 149 µg/L (daily mean concentrations up to 30 µg/L) exhibited survival rates of 90% or greater during this experiment. Of the steelhead exposed to a peak of 263 µg/L (mean of 51), 32% survived. Very few embryos survived to the alevin stage when exposed to daily peaks of 465 µg/L (mean of 109), and all had died before the 8th wk of exposure.

The relationship between mean copper concentration and survival was examined at the 7th wk, before any group had been eliminated by mortality. The expected sigmoid shape of these curves was not well defined in the lower portion, and the survival curve (Fig. 2) fitted by eye may not adequately represent the lower part. Above concentrations of about 30 µg/L survival was greater for developing steelhead exposed continuously rather than intermittently when exposure was expressed as mean concentration. When both exposure regimens were expressed as maximum or peak concentrations, intermittently exposed steelhead understandably had greater survival than those continuously exposed at the same maximum concentration.

Growth and Development

Fifty percent of embryos exposed continuously to control water or copper solutions at mean copper concentrations of 32 µg/L or lower hatched on day 31 followed fertilization. Time to 50% hatching was 32 d at 59 µg/L and 33 d at 124 µg/L. Intermittently exposed embryos hatched in 32 d at mean concentrations of 16 µg/L or lower and in 33 days at mean concentrations of 30 µg/L or higher. All intermittently exposed groups and those continuously exposed groups at mean concentrations greater than 32 µg/L hatched 1 or 2 d later than controls and low concentration continuous exposure groups.

Mean dry weights of copper-exposed steelhead were significantly less at hatching than controls at the highest continuous concentrations and the two highest intermittent concentrations. Marked concentration-dependent depression of growth appeared as the test progressed and could be at least partially attributed to decreased food consumption (Table 1). Measurement of unconsumed ration was not attempted due to the small particle size, but residual food was regularly observed only in the tanks of fish that exhibited low body weights. Final dry weights demonstrated the greater reduction in fish size with increasing copper concentration for fish exposed intermittently than for those exposed continuously (Fig. 3). The size difference appeared to be greatest in the intermediate concentration. When concentrations producing 50% reduction in final dry weights were calculated (EC50's), intermittent exposure was approximately twice as damaging as continuous exposure. Mean EC50 values of 27 and 46 µg/L were obtained for intermittent and continuous exposures, respectively.

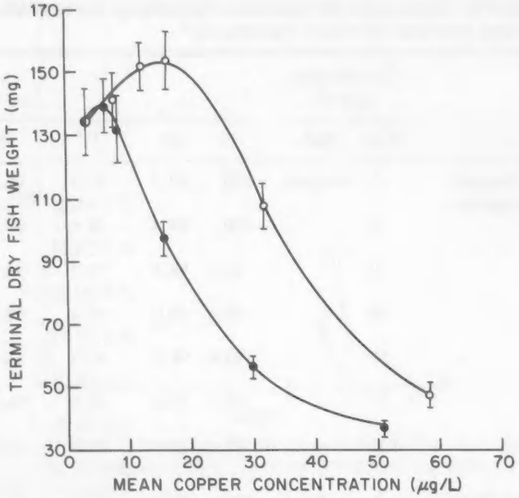

FIG. 3. Comparison of the mean dry weight of steelhead alevins ($N = 20$) following 78 d of intermittent or continuous copper exposure. Concentrations are expressed in terms of the mean copper concentrations measured in exposure water throughout the experiment. ○, continuous exposure; ●, intermittent exposure; ◐, control.

Growth rates from hatching to day 78 of the experiment were influenced by the size at hatching, the time of hatching, and the final size attained. Instantaneous growth rates at mean concentrations beyond about 15 µg/L, calculated for the 55- to 57-d period from hatching to day 78, were generally less for steelhead exposed intermittently than those exposed continuously. Only fish exposed intermittently to the lowest concentration grew faster than control and continuously exposed steelhead; however, because of their slightly smaller size at hatching and later time of hatching, these faster growing fish were still smaller at termination than comparable continuously exposed fish.

Copper Accumulation

Efficiency of copper residue analysis was greater than 95% (i.e. range of 95–103% recovery in bovine liver standard). Whole-alevin copper concentrations determined at two time points indicated concentration-dependent bioaccumulation (Fig. 4). The data were described by a set of four parallel lines on a log–log plot ($r = 0.90$ for the data set). At both times, intermittent exposure produced greater copper bioaccumulation than continuous exposures producing the same daily mean exposure concentrations. The arithmetic relationship of fish dry weight versus whole body copper concentration was linear ($r \geq 0.92$ in each case). While separate nonparallel lines were determined for the two times, differences at either sampling time were not related to the type of exposure (Fig. 5). Thus, while accumulation was affected by temporal aspects of

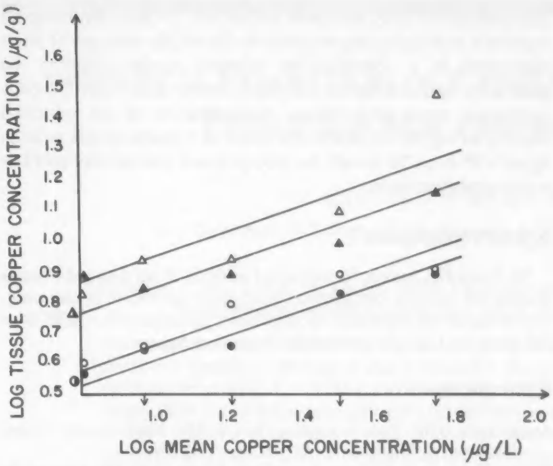

FIG. 4. Log–log plot of mean exposure water concentration versus whole dry fish copper concentration after approximately 8 and 11 wk of treatment. Fitted lines are parallel ($r = 0.90$) for the data set. ●, April continuous; ○, April intermittent; ▲, May continuous; △, May intermittent.

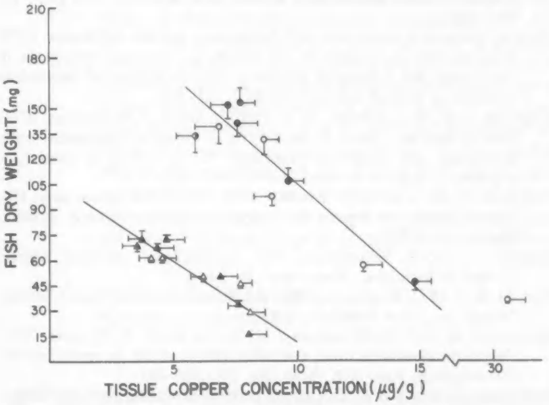

FIG. 5. Levels of tissue copper (μg/g dry wt) in whole fish as a function of body weight ($N = 10$) after approximately 8 and 11 wk of treatment. Fitted lines are nonparallel with significantly different y-intercepts. Points are means ± SE, $r \geq -0.92$. ▲, April continuous; △, April intermittent; ◭, April control; ●, May continuous; ○, May intermittent; ◑, May control.

exposure, the relationship between body burden of metal and fish size was not different between intermittent and continuous exposures.

Acute Mortality

The 96-h LC50 determined for fish from the same cohort as the test group was 80 μg Cu/L for continuous exposure. Intermittent exposure LC50 test results were unsatisfactory, with peak copper concentrations of 138, 200, 330, and 490 μg/L producing mortality at 0, 0, 90, and 60%, respectively. A log–probit graphical estimate of the intermittent LC50 was about 360 μg/L peak concentration (mean 76 μg/L) for 1.1 g of fish.

Discussion

Development of appropriate water quality criteria is limited by the lack of understanding of relationships between durations and concentrations of toxicant exposures and the effects of these exposures on aquatic organisms. Such information is particularly important because aquatic organisms in receiving waters are often chronically exposed to temporally variable concentrations of environmental contaminants, not the continuous levels employed in most laboratory testing.

The establishment of relationships between onset of toxic responses and the toxicant concentrations in environmental air of mice was studied by a group of American and Russian scientists (Coffin et al. 1977; Gardner et al. 1977). Time to response–concentration relationships for continuous exposures were linear on log–log plots. This pattern was observed with nitrogen dioxide, sulfur dioxide, or benzene and seven toxic responses. When intermittent schedules of toxicant exposure were employed, variations of exposure concentration and duration with equivalent cumulative concentrations produced relationships that varied between toxicants. At given benzene concentrations, the time required to produce an effect was greater for an intermittent than continuous exposure. This was interpreted as recovery between pulses of benzene in the intermittent situation. Recovery between pulses of nitrogen dioxide was not observed. When the product of concentration and time was based on a 24-h average, more marked toxic effects were caused by intermittent than continuous exposures. They concluded that short-term peak concentrations must be considered when maximum allowable concentrations are established.

Our results demonstrated the tenuous nature of applying toxicity data from continuous exposure studies for estimates of intermittent exposure responses. When either survival or growth was followed through time (Table 1), mean copper concentrations were more toxic following intermittent than continuous exposures. Percent survival at 7 wk postfertilization (Fig. 2) and dry weight of alevins at 8 wk posthatching (Fig. 3) both indicated that intermittent exposure was much more toxic to fish than continuous treatment at the same mean concentrations. A 30% inhibition of fish growth occurred at a mean copper concentration of 16 μg/L for intermittent but not continuous exposure (Fig. 3). The peak concentration (76 μg/L) for this intermittent exposure group approximated the 96-h LC50 for steelhead juveniles and therefore might be expected to produce sublethal toxicity.

The proposal (Anonymous 1980) that daily maximum toxicant concentrations be permitted to equal the 96-h LC50 if the daily average does not exceed the chronic threshold concentration (the geometric mean of the lowest effect level and the highest no-effect level) was evaluated using the data generated in this study. Standard continuous exposure toxicity tests yielded a 96-h LC50 value of 80 μg/L and a chronic threshold value of 22 μg/L (the geometric mean of the lowest effect level of 31 μg/L and the highest no-effect level of 16 μg/L). Therefore, a daily maximum concentration of 80 μg/L and a 24-h mean concentration of 22 μg/L would represent a projected no-effect exposure.

Based on this estimation, the intermittent exposure with a peak of 76 μg/L (mean 16 μg/L) presumably should have had no effect. In fact, this treatment reduced final weight by about 30%. The next lower concentration (peak 35 μg/L, mean 8 μg/L) produced no observed adverse effect. The estimated threshold chronic value based directly on the intermittent exposure results and expressed as the geometric mean of 76 and 35 µg/L was 52 µg/L peak and 11 µg/L as the 24-h mean. These calculations indicate that an exposure regimen composed of a daily maximum of one-half the continuous 96-h LC50 and a daily mean of one-half the continuous chronic threshold value would have been adequately protective.

Different dosing patterns might produce other relationships between intermittent and continuous exposures, but based on this study the proposal to use acute LC50 values as acceptable daily maxima and chronic threshold estimates as acceptable 24-h mean concentrations appears to be underprotective by about a factor of 2. More studies will be required to determine the general applicability of these findings. For some purposes, agreement within a factor of 2 may be acceptable or within the bounds of experimental, analytical, and natural variation. Certainly the proposed criteria expressions for peak and 24-h mean concentrations based on continuous exposure tests (Anonymous 1980) are not overprotective for regularly cycling exposures of the sort used in this study.

Copper bioaccumulates in fish under field conditions (Chernoff and Dooley 1979). In our test, intermittent exposures produced higher tissue copper concentrations than continuous treatments at equivalent daily mean concentrations (Fig. 4). Further, when dry weight of fish was regressed against whole body copper concentration, a strong negative correlation was found (Fig. 5). Differences in toxicity between exposure regimens were therefore correlated with copper bioaccumulation. The degree of toxicity apparently was related to the toxicant concentration in the fish, regardless of exposure pattern. The operative mechanisms of the time–concentration relationships of toxicant exposure remain obscure. For intermittent exposures, the magnitude of the concentration gradient of copper between the fish and surrounding water was much greater than for equivalent mean continuous exposures. Fick's Law predicts that flux of nonionized substances is directly proportional to the concentration gradient for passive transport (Notari 1975). Since copper speciation was not determined in our experiments, direct application of this principle to our data was precluded. Also, during intervals of high copper concentration, endogenous protective mechanisms may have been overwhelmed. Interaction of these factors probably contributed to differences in both toxicity and accumulation of copper between exposure regimens.

Calculation of exposure units (micrograms of copper per litre per hour) was useful in determining mean concentrations but revealed no additional insight as a dose expression. This was expected, since they are directly proportional to means. How various time–concentration interactions affect the toxicity of given agents probably requires case by case consideration, although generalizations for appropriate classes of compounds may emerge as more toxicants are tested. A carefully designed approach examining representative chemicals selected by their placement in a classification scheme would optimize the generality and usefulness of comparative data. Until such a systematic approach evolves, consideration of the potential toxicity of repetitive short exposures to concentrations near an agent's 96-h LC50 should be incorporated into development of water quality criteria.

Acknowledgments

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Empirical Prediction of Crustacean Zooplankton Biomass and Profundal Macrobenthos Biomass in Lakes[1]

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Hanson, J. M., and R. H. Peters. 1984. Empirical prediction of crustacean zooplankton biomass and profundal macrobenthos biomass in lakes. Can. J. Fish. Aquat. Sci. 41: 439–445.

We used data taken from the literature to develop and compare several estimators of crustacean zooplankton biomass (49 lakes) and profundal macrobenthos biomass (38 lakes). Both mean zooplankton biomass ($r^2 = 0.72$, $P < 0.001$) and mean profundal macrobenthos biomass ($r^2 = 0.48$, $P < 0.001$) correlated better with mean total phosphorus concentration than with Secchi depth, mean depth, maximum depth, or lake surface area. Mean total phosphorus concentration was also superior to mean chlorophyll *a* concentration ($r^2 = 0.57$, $P < 0.001$) as an estimator of zooplankton biomass, but data were insufficient to evaluate chlorophyll a concentration as an estimator of macrobenthos biomass. Inclusion of maximum depth as a variable in a multiple regression resulted in a slight but significant ($P < 0.030$) improvement in the zooplankton–total phosphorus relationship ($R^2 = 0.75$, $P < 0.001$). Inclusion of lake surface area as a variable in a multiple regression significantly ($P < 0.001$) improved the predictive power of the profundal macrobenthos–total phosphorus relationship ($R^2 = 0.59$, $P < 0.001$).

À partir de données extraites de travaux publiés, nous avons mis au point et comparé plusieurs estimateurs de la biomasse de crustacés zooplanctoniques (49 lacs) et de celle du macrobenthos de profondeur (38 lacs). La biomasse moyenne du zooplancton ($r^2 = 0{,}72$, $P < 0{,}001$) et celle du macrobenthos de profondeur ($r^2 = 0{,}48$, $P < 0{,}001$) montrent toutes deux une meilleure corrélation avec la concentration de phosphore total qu'avec la profondeur du disque de Secchi, la profondeur moyenne, la profondeur maximale ou la superficie du lac. La concentration moyenne de phosphore total est aussi supérieure à la concentration moyenne de chlorophylle *a* ($r^2 = 0{,}57$, $P < 0{,}001$) comme estimateur de la biomasse zooplanctonique, mais les données ne nous permettent pas d'évaluer la concentration de chlorophylle *a* comme estimateur de la biomasse de macrobenthos. Si l'on inclut la profondeur maximale comme variable dans une régression multiple, il en résulte une amélioration faible mais significative ($P < 0{,}030$) de la relation zooplancton–phosphore total ($R^2 = 0{,}75$, $P < 0{,}001$). L'inclusion de la superficie du lac comme variable dans une régression de ce genre améliore de façon significative ($P < 0{,}001$) le pouvoir prévisionnel de la relation macrobenthos de profondeur–phosphore total ($R^2 = 0{,}59$, $P < 0{,}001$).

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One goal of fisheries managers and limnologists is to predict the effects of changing nutrient levels on lake biomass and productivity. In recent years, total phosphorus concentration has been related empirically to chlorophyll *a* concentration (Dillon and Rigler 1974; Nicholls and Dillon 1978; Prepas and Trew 1983), phytoplankton biomass (Nicholls and Dillon 1978; Schindler et al. 1978; Kalff and Knoechel 1978), fish biomass (Hanson and Leggett 1982), and fish yield (Hanson and Leggett 1982; Jones and Hoyer 1982) for predictive purposes. Fish yield is also strongly correlated with chlorophyll *a* concentration (Oglesby 1977; Jones and Hoyer 1982). Relatively few correlations of zooplankton biomass or macrobenthos biomass with indices of lake trophic status have been reported.

McCauley and Kalff (1981) described strong predictive relationships between crustacean zooplankton biomass and phytoplankton biomass based on 207 individual observations within 13 lakes ($r^2 = 0.40$) and on average values for 15 European and 2 North American lakes ($r^2 = 0.72$). Mills and Schiavone (1982) reported significant correlation of crustacean zooplankton biomass with chlorophyll *a* concentration ($r^2 = 0.56$, $P < 0.001$) and Secchi depth ($r^2 = 0.41$, $P < 0.001$) for 13 New York lakes but did not report the regression equations. Our further analysis of their data revealed strong linear correlation of zooplankton biomass with total phosphorus concentration ($r^2 = 0.71$, $P < 0.01$) and total dissolved solids ($r^2 = 0.53$, $P < 0.01$). In contrast, Yan and Strus (1980) did not find significant correlation of crustacean zooplankton biomass with total phosphorus concentration ($P > 0.10$) or phytoplankton biomass ($P > 0.05$) for 21 observations from acidic and nonacidic lakes in Ontario. Bays and Crisman (1983) found only a weak correlation ($r^2 = 0.15$, $P < 0.02$) of crustacean zooplankton biomass with Carlson's trophic state index (based on chlorophyll *a* concentration) for 39 Florida lakes.

Even fewer researchers have attempted to predict macrobenthos biomass in lakes. Rawson (1952) reported a significant inverse relationship of macrobenthos biomass and mean depth in 12 northern Saskatchewan lakes, but Hayes (1957) did not find a significant relationship when the data set was expanded to

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[1] A contribution of the Lake Memphremagog Project, Limnology Research Center, McGill University.

TABLE 1. Geometric means and range of mean zooplankton biomass (ZB in mg/m^3, dry wt), mean profundal macrobenthos biomass (PMB in g/m^2, wet wt), mean total phosphorus concentration (TP in mg/m^3), mean chlorophyll *a* concentration (CHLA in mg/m^3), Secchi depth (SECC in m), mean depth (ZME in m), maximum depth (ZMAX in m), and lake surface area (AREA in km^2) derived from the literature.

	ZB	PMB	TP	CHLA	SECC	ZME	ZMAX	AREA
				Zooplankton data set (N = 49)				
Mean	88.0	—	20.4	8.9	3.1	11.2	28.7	14.83
Range	10.7–786.0	—	4.0–200.0	0.4–300.0	0.5–28.0	1.3–177.4	1.8–397.0	0.04–82 400.0
				Profundal macrobenthos data set (N = 38)				
Mean	—	6.1	9.5	—	5.5	12.5	32.9	13.68
Range	—	1.0–52.5	2.7–117.0	—	1.5–23.0	2.9–148.0	7.0–397.0	0.18–82 400.0

include European and additional North American lakes. Experimental studies have shown that littoral macrobenthos biomass increased following nutrient addition to a small lake (Smith 1969) and to small artificial ponds (Hall et al. 1970). Dermott et al. (1977) reported that sublittoral macrobenthos biomass increased with total phosphorus concentration along a nutrient gradient in Lake Memphremagog, Quebec–Vermont.

The purpose of this study was to compare systematically the relative predictive power of several correlates of crustacean zooplankton biomass over a wide range of lakes from different geographic areas and to develop and evaluate empirical estimators of profundal macrobenthos biomass in lakes.

Data and Analyses

Crustacean Zooplankton Biomass

The data set consisted of 33 North American, 14 European, and 2 African lakes for which mean total phosphorus concentration (mg/m^3), mean chlorophyll *a* concentration (mg/m^3), Secchi depth (m), mean depth (m), maximum depth (m), lake surface area (km^2), and mean crustacean zooplankton biomass (mg/m^3, dry wt) were reported. Lakes with pH below 5.5 were not included because low pH appears to reduce zooplankton diversity and biomass (Sprules 1975; Yan and Strus 1980; Confer et al. 1983). The geometric means and ranges of reported values for the zooplankton data set are shown in Table 1.

Mean total phosphorus concentration was reported for the epilimnion for 36 lakes and the sampling depth was not specified for the remaining 13 lakes. Mean chlorophyll *a* concentration was measured in the epilimnion in 43 lakes and the depth was not specified for 6 lakes. Most studies did not state whether chlorophyll *a* concentrations were corrected for phaeopigments. Total phosphorus and chlorophyll *a* were sampled at weekly (20 lakes), biweekly (4 lakes), or monthly (11 lakes) intervals. Mean total phosphorus and chlorophyll *a* concentrations represent the ice-free period (28 lakes) or summer months only (19 lakes). Means based on two or more years of data were reported for 20 lakes.

Crustacean zooplankton biomass was measured for the whole water column (46 lakes), to 30 m (1 lake), to 50 m (2 lakes), or to 60 m (1 lake). Zooplankton were sampled by vertical tows (17 lakes of which only five data sets were corrected for sampling inefficiency) or tubes and traps (22 lakes). The net mesh was 30–80 μm (26 lakes) or 105–153 μm (14 lakes). Zooplankton were sampled at weekly (19 lakes), biweekly (5 lakes), or monthly (19 lakes) intervals. Means based on two or more years of sampling were reported for 19 lakes. Lakes were sampled during the ice-free season (41 lakes) or during summer months only (6 lakes). Zooplankton wet weights, in the few instances where reported, were converted to dry weights using a dry weight to wet weight ratio of 0.120 (Taguchi and Fukuchi 1975; McCauley and Kalff 1981).

The predictive ability of the total phosphorus and chlorophyll *a* concentration models were tested with a further data set not included in the original analyses. This data set contained mean epilimnetic total phosphorus concentration, mean epilimnetic chlorophyll *a* concentration (corrected for phaeopigments), and mean crustacean zooplankton biomass sampled monthly from May until September for six lakes in the Eastern Townships region of Quebec. Triplicate zooplankton samples were taken by corrected vertical tow or by a bilge pump. In both cases, crustacean zooplankton were retained by 75-μm mesh (M. L. Pace, Department of Oceanography, University of Hawaii, Honolulu, HA 96822, pers. comm.).

Profundal Macrobenthos Biomass

The data set (means and ranges in Table 1) consisted of 31 North American, 5 European, and 2 New Zealand lakes for which mean total phosphorus concentration (mg/m^3), Secchi depth (m), mean depth (m), maximum depth (m), lake surface area (km^2), and mean profundal macrobenthos biomass (g/m^2, wet wt) were reported. Reservoirs were not included because water level fluctuations, oxygen depletions, and heavy siltation severely reduce macrobenthos biomass (Cowell and Hudson 1967; Isom 1971; Nagiec 1977). We only used profundal macrobenthos biomass because methods used to estimate littoral zone macrobenthos biomass vary greatly and do not give equivalent results (Gerking 1957; Mackie and Quadri 1971; Keast 1978). Mean chlorophyll *a* concentration could not be evaluated because it was not reported for 11 lakes and the remaining lakes did not vary greatly in chlorophyll level. The macrobenthos biomass data set had only seven lakes in common with the zooplankton biomass data set.

Total phosphorus concentration was measured in the epilimnetic waters for all 32 lakes where methods were reported. Total phosphorus was measured at weekly (1 lake), biweekly (16 lakes), or monthly (11 lakes) intervals.

Profundal macrobenthos were defined as invertebrates recovered from sediment samples taken from depths greater than the epilimnion, excluding copepods, cladocerans, ostracods, nematodes, coelenterates, and water mites. Profundal macrobenthos samples were taken at discrete sampling stations and/or along transect lines in the deepest part of the lakes using Ekman (30 lakes), Peterson (1 lake), or Ponar dredges (1 lake). Sediments were sifted through sieves of 670-μm (11 lakes),

TABLE 2. Regression equations for predicting zooplankton biomass (Y in mg/m^3, dry wt) from total phosphorus concentration (TP in mg/m^3), chlorophyll *a* concentration (CHLA in mg/m^3), Secchi depth (SECC in m), mean depth (ZME in m), maximum depth (ZMAX in m), and lake surface area (AREA in km^2).

	r^2	P^a	SE of slope
Simple regressions			
1. log Y = 0.907 log TP + 0.756	0.72	0.001	0.082
2. log Y = 0.529 log CHLA + 1.44	0.57	0.001	0.067
3. log Y = −0.878 log SECC + 2.38	0.59	0.001	0.106
4. log Y = −0.487 log ZME + 2.46	0.30	0.001	0.110
5. log Y = −0.484 log ZMAX + 2.65	0.35	0.001	0.096
6. log Y = 0.015 log AREA + 1.93	0.00	0.724	0.043
7. log CHLA = 1.25 log TP − 0.676	0.66	0.001	0.133
Multiple regressions			
8. log Y = 0.979 log TP − 0.158 log ZMAX + 1.13,	$R^2 = 0.75$,	$P^a < 0.001$	
9. log Y = 0.430 log CHLA − 0.243 log ZMAX + 1.89,	$R^2 = 0.64$,	$P^a < 0.001$	
10. log Y = − 0.528 log SECC + 0.283 log CHLA + 1.94,	$R^2 = 0.66$,	$P^a < 0.001$	

[a] *F*-test.

500-µm (1 lake), 400-µm (18 lakes), or 250-µm (1 lake) mesh before sorting. Lakes were sampled monthly (27 lakes) or biweekly (3 lakes) during the ice-free season (28 lakes), summer months only (2 lakes), or in spring and fall (2 lakes). Nineteen lakes were sampled for two or more years.

We did not attempt to evaluate total dissolved solids or the morphoedaphic index (total dissolved solids/mean depth, Ryder 1965) as estimators of crustacean zooplankton biomass or profundal macrobenthos biomass because total dissolved solids data were not available for all lakes in either data set.

Significant ($P < 0.050$) predictive relationships for mean crustacean zooplankton biomass and mean profundal macrobenthos biomass data sets were detected by least squares methods of linear regression and stepwise multiple regression. Intercorrelation between variables was detected by Pearson product-moment correlation. All data were $\log_{10}$-transformed to stabilize the variance, to distribute the data more evenly, and to linearize the data. All analyses used the SAS statistical package.[2]

Results

Crustacean Zooplankton Biomass

Mean total phosphorus concentration was the strongest ($r^2 = 0.72$, Fig. 1) univariate estimator of mean crustacean zooplankton biomass (Table 2). The slope of the zooplankton biomass–total phosphorus relationship did not differ significantly from 1.0, which suggests that over the range of values studied, zooplankton biomass varied in direct proportion to total phosphorus concentration. Zooplankton biomass was inversely related to Secchi depth ($r^2 = 0.59$). The zooplankton biomass–chlorophyll *a* concentration was equally strong ($r^2 = 0.57$) but the slope was significantly less than unity. Zooplankton biomass was inversely correlated with mean depth ($r^2 = 0.30$) and maximum depth ($r^2 = 0.35$) but was not correlated with lake surface area ($P = 0.724$). Secchi depth and chlorophyll *a* concentration were strongly correlated ($P < 0.001$) with total phosphorus concentration ($r = -0.79$ and $r = 0.82$, respectively) and with each other ($r = -0.76$). Stepwise multiple regression analyses showed that maximum depth was the only independent variable to significantly ($P < 0.030$) improve the predictive power of the zooplankton biomass–total phosphorus concentration model ($R^2 = 0.75$, Table 2). Multiple regressions including chlorophyll *a* concentration and maximum depth ($R^2 = 0.64$) or Secchi depth and chlorophyll *a* concentration ($R^2 = 0.66$) as variables also explained a large fraction of the variation in mean zooplankton biomass. Mean depth and lake surface area did not improve the predictive power of any of the models tested.

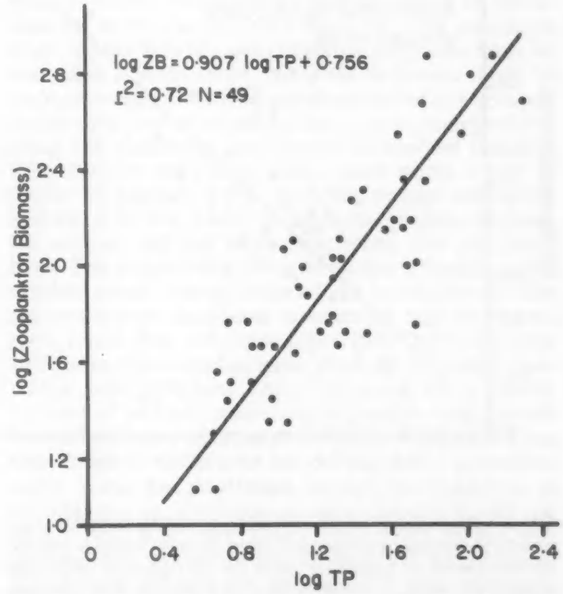

FIG. 1. Regression of zooplankton biomass (ZB in mg/m^3, dry wt) on total phosphorus concentration (TP in mg/m^3). Statistics required for the construction of confidence limits for the regression (Sokal and Rohlf 1969) are $S^2_{y \cdot x} = 0.056$, $\Sigma x^2 = 8.4319$, $S_b = 0.0817$, and $\bar{X} = 1.3096$.

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[2] Complete data sets used in these analyses and their sources are available, at a nominal cost, from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Canada K1A 0S2.

TABLE 3. Regression equations for predicting profundal macrobenthos (Y in g/m^2, wet wt) from total phosphorus concentration (TP in mg/m^3), Secchi depth (SECC in m), mean depth (ZME in m), maximum depth (ZMAX in m), and lake surface area (AREA in km^2).

Simple regressions			
1. log Y = 0.708 log TP + 0.092	0.48	0.001	0.124
2. log Y = −0.852 log SECC + 1.41	0.35	0.001	0.194
3. log Y = −0.328 log ZMAX + 1.28	0.15	0.016	0.130
4. log Y = −0.325 log ZME + 1.14	0.15	0.015	0.128
5. log Y = −0.069 log AREA + 0.862	0.06	0.136	0.045
Multiple regressions			
6. log Y = 0.742 log TP − 0.090 log AREA + 0.161,	$R^2 = 0.59$,	$P^a < 0.001$	
7. log Y = 0.653 log TP − 0.216 log ZME + 0.383,	$R^2 = 0.54$,	$P^a < 0.001$	
8. log Y = 0.653 log TP − 0.215 log ZMAX − 0.382,	$R^2 = 0.54$,	$P^a < 0.001$	

[a] *F*-test.

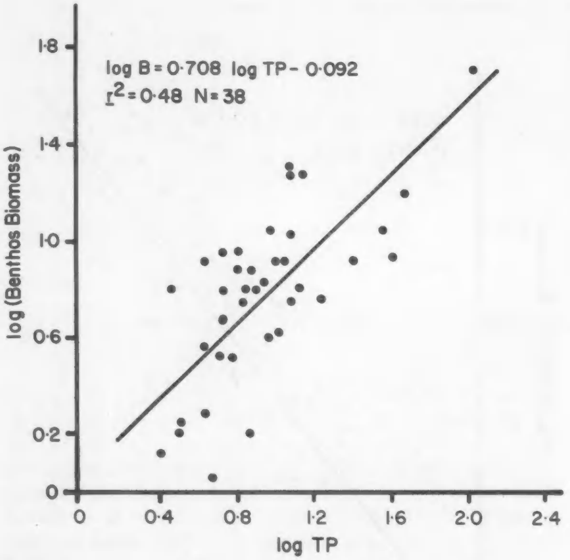

FIG. 2. Regression of macrobenthos biomass (PMB in g/m^2, wet wt) on total phosphorus concentration (TP in mg/m^3). Statistics required for construction of confidence limits for the regression (Sokal and Rohlf 1969) are $S^2_{y\cdot x} = 0.0696$, $\Sigma x^2 = 4.549$, $S_b = 0.1238$, and $\bar{X} = 0.9777$.

Profundal Macrobenthos Biomass

Mean epilimnetic total phosphorus concentration was also the strongest univariate estimator of mean profundal macrobenthos biomass ($r^2 = 0.48$, Fig. 2). Despite the wide scatter about the line, the slope of the relationship was significantly less than unity, which suggest that profundal macrobenthos biomass did not rise as rapidly as total phosphorus concentration over the range of data studied. Profundal macrobenthos biomass showed a moderately strong inverse relationship with Secchi depth ($r^2 = 0.35$, Table 3) and weak inverse relationships with mean depth ($r^2 = 0.15$) and maximum depth ($r^2 = 0.15$). Profundal macrobenthos biomass was not significantly correlated with lake surface area ($P = 0.136$). Stepwise multiple regression analyses showed that inclusion of lake surface area as an independent variable significantly improved ($P < 0.001$) the predictive power of the profundal macrobenthos biomass–total phosphorus concentration model ($R^2 = 0.59$). Furthermore, analysis of residuals suggested a slight bias in the simple profundal macrobenthos–total phosphorus model. This bias was no longer apparent in the multiple regression on total phosphorus concentration and lake surface area. Mean depth and maximum depth, both of which were positively correlated with lake surface area ($r = 0.68$ and $r = 0.77$, respectively), equally improved the macrobenthos biomass–total phosphorus relationship ($R^2 = 0.54$, Table 3). Secchi depth did not significantly improve the predictive power of the macrobenthos biomass–total phosphorus model nor did additional variables improve the macrobenthos biomass–Secchi depth model. Total phosphorus concentration was not significantly correlated with lake surface area ($P > 0.500$), mean depth ($P > 0.200$), or maximum depth ($P > 0.400$).

Discussion

Mean total phosphorus concentration was superior to mean chlorophyll *a* concentration, Secchi depth, mean depth, maximum depth, or lake surface area as an estimator of mean crustacean zooplankton biomass when comparisons were based on the same data set. Total phosphorus concentration, chlorophyll *a* concentration, and Secchi depth are interrelated indices of lake trophy (Sakamoto 1966; Carlson 1977; Canfield and Bachmann 1981; this study), and all correlations therefore indicate that richer lakes support greater zooplankton biomass. Total phosphorus concentration is likely the best correlate because transparency and chlorophyll *a* concentration are more variable over the seasons than total phosphorus concentration. This greater variation is expected for chlorophyll *a* concentration because the exponent of the chlorophyll *a*–total phosphorus relationship is generally significantly greater than unity (Sakamoto 1966; Dillon and Rigler 1974; Jones and Bachmann 1976; this study), which indicates that a unit increase in total phosphorus concentration results in a proportionately greater increase in chlorophyll *a* concentration.

If total phosphorus concentrations are unavailable, multiple regression models based on mean chlorophyll *a* concentration and maximum depth or on Secchi depth and chlorophyll *a* concentration also explain a large part of the variation in zooplankton biomass. Because chlorophyll *a* concentration and Secchi depth are themselves highly correlated (Barica 1975; Dillon and Rigler 1975; Nicholls and Dillon 1978; Canfield and

TABLE 4. Comparison of predicted (PZB) and observed (OZB) values of zooplankton biomass (mg/m^3, dry wt) based on total phosphorus concentration (TP) and chlorophyll *a* concentration (CHL) models. Zooplankton biomass estimates were corrected for back-transformation (Baskerville 1972; Sprugel 1983); 95% confidence intervals of the estimates (CI) and % observed biomass (% OZB) are presented.

				TP model				CHL model			
Lake	OZB	TP	CHL[a]	−CI	PZB	+CI	% OZB	−CI	PZB	+CI	% OZB
Brompton	44.6	6.2	1.9	11.3	34.6	105.9	77.6	12.2	49.1	196.4	110.1
Lovering	58.9	7.0	2.5	12.6	38.6	117.8	65.5	14.2	56.7	226.1	96.3
Memphremagog											
North Basin	103.7	9.3	3.6	16.4	50.0	151.7	48.2	17.4	68.6	272.8	66.3
Quinn Bay	76.5	10.0	4.5	17.6	53.4	161.8	69.8	19.6	77.4	306.2	101.2
South Basin	97.8	18.4[b]	6.6	30.8	92.8	279.5	94.9	24.0	94.8	374.1	96.9
Brome	143.1	14.4	3.5	24.6	74.3	224.1	51.9	17.1	67.8	268.9	47.4
Magog	233.9	47.0	15.5	71.6	217.4	660.2	92.9	37.7	148.9	588.5	63.7
Waterloo	243.3	56.0	28.6	83.6	254.8	776.7	104.7	51.7	205.9	819.3	84.6

[a] Corrected for phaeopigments.

[b] From Janus and Vollenweider (1981).

Bachmann 1981) the regression coefficients on both variables in the multiple regressions have higher standard errors and their independent effects cannot be estimated. Nevertheless, the relationships can be used for prediction when the colinearity between independent variables can be expected in future application (Gujarati 1978). This is certainly the case for chlorophyll *a* concentration and Secchi depth.

Our zooplankton biomass–total phosphorus concentration model (Table 2) explains a similar proportion of the variance in zooplankton biomass ($r^2 = 0.72$) as the zooplankton biomass–phytoplankton biomass model of McCauley and Kalff (1981). The exponent of the zooplankton biomass–total phosphorus relationship did not differ significantly from unity whereas the exponent of the zooplankton biomass–phytoplankton biomass model was significantly smaller. These two models are consistent only if phytoplankton biomass increases more rapidly than total phosphorus concentration. Kalff and Knoechel (1978) reported the exponent of their phytoplankton biomass–total phosphorus concentration model to be 1.209. Nicholls and Dillon (1978) and Schindler et al. (1978) described a linear increase in phytoplankton biomass over about a fivefold range in total phosphorus concentration. However, because the intercept on the ordinate (phytoplankton biomass) is negative, the ratio of phytoplankton biomass to total phosphorus concentration increases in eutrophy. This faster increase in phytoplankton volume might be expected because average algal size increases in eutrophy (Watson and Kalff 1981) and larger algal cells appear to have lower phosphorus concentrations, at least in phosphorus-limited systems (Shuter 1978). These trends show that the models predicting zooplankton biomass (McCauley and Kalff 1981; this study) are at least in qualitative agreement although it is clearly easier, from a practical standpoint, to measure total phosphorus concentration.

We compared the predictive ability of our models based on total phosphorus concentration and chlorophyll *a* concentration in a further, independent data set (Table 4). Both models yielded similar predictions of zooplankton biomass (47–110% of observed biomass levels), but the 95% confidence intervals around predictions based on total phosphorus had a smaller range (about 9-fold) than those based on chlorophyll (about 16-fold). Using their test data, we recalculated zooplankton biomass (corrected for back transformation, Baskerville 1972; Sprugel 1983) predicted from the zooplankton biomass–phytoplankton biomass model (McCauley and Kalff 1981): the predicted zooplankton biomass was 31–121% of observed biomass, but the 95% confidence intervals around the predictions had a 15.7- to 26.7-fold range. The total phosphorus model appears to have greater predictive utility than either the chlorophyll *a* model or the phytoplankton biomass model. A further advantage of the total phosphorus model is that average total phosphorus concentration is usually tightly correlated with spring total phosphorus concentration (Chapra and Tarapchak 1976; Nicholls and Dillon 1978), which means it may be possible to construct models predicting average zooplankton biomass, or for that matter, profundal macrobenthos biomass, fish biomass, and fish yield, using spring total phosphorus levels. This would greatly reduce the number of samples needed to predict animal biomass in lakes. Since models now exist that can predict mean phosphorus concentration from topographic maps, census data, and climatological data (Dillon and Rigler 1975), even this minimal sampling effort may be circumvented.

Mean total phosphorus concentration was also a superior estimator of profundal macrobenthos biomass compared with Secchi depth, mean depth, maximum depth, or lake surface area when compared for the same data set. The greater unexplained scatter in the data may reflect, in part, the dependence of macrobenthos on allochthonous energy sources (Louks and Odums 1978; Rau 1980; Timms 1980) which are less well represented by lake phosphorus concentration. Recent experimental work demonstrates that increased input of organic material to lakes results in increased macrobenthic invertebrate populations (Danell and Andersson 1982). The importance of allochthonous material as a regulator of macrobenthic biomass ought to decline with lake surface area, a hypothesis supported by the negative effect of lake surface area on profundal macrobenthos biomass in our multiple regression with total phosphorus concentration (Table 3). Unfortunately, the importance of allochthonous input for macrobenthos production, among lakes, is difficult to evaluate. Further work is warranted because macrobenthos are thought to be the primary route of energy transfer in lakes (Efford 1969; Jonasson 1978; Wissmar and Wetzel 1978; Eggers et al. 1978).

Although the models presented are relatively powerful and simple, residual variation is high. A part of this variation is likely methodological. Sampling methods and sampling schedules may be inadequate to represent mean seasonal biomass, mean total phosphorus concentration, or mean chlorophyll *a* concentration. Zooplankton biomass estimates, in particular, may be inadequate because most researchers rely on a single vertical sample at a single sample site, ignoring the possibility of horizontal heterogeneity of zooplankton abundance. If models to predict zooplankton biomass and profundal macrobenthos biomass are to be more accurate, they may require more precise data. Further improvement may be achieved by extending the range of data to test the applicability of the models, by examining additional variables to improve the predictive power of the models (Rigler 1982; Peters 1983), and by improving and standardizing techniques used to measure both dependent and independent variables.

Mean total phosphorus concentration appears to be a useful general index of lake trophy. With the exception of fish biomass and yield, however, these correlations apply only to open-water or profundal biomass. The littoral zone is believed to be the most productive area of lakes (Anderson and Hooper 1956; Welch and Kalff 1974; Jonasson 1978; Kajak and Dusoges 1976), yet reliable estimates of littoral zone animal biomass are scarce and much work is required before correlations of littoral zone production to lake nutrient levels or morphometry can be attempted. The measurement of nonlittoral zone biomass remains useful because it, potentially, can be related to valuable coregonid and salmonid fish populations that generally inhabit the offshore waters of lakes (Grimaldi 1972; Scott and Crossman 1973; Gascon and Leggett 1977).

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Optimal versus Unregulated Industry Behavior in a Beverton–Holt Multicohort Fishery Model

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Canadian government policy toward fisheries in recent years has shifted emphasis from maximizing the sustainable catch level toward maximizing economic yield taking account of fishing costs and revenues. I have provided a model for the determination of the optimal catch rate and mesh size for maximum economic yield in a Beverton–Holt model employing many age cohorts. The optimal effort and mesh size are compared with those predicted for an unregulated fishery. While the analysis is mainly limited to steady state, an attempt is also made to determine the optimal disequilibrium response to environmentally caused exogenous changes in recruitment.

La politique des pêches du gouvernement canadien a évolué ces dernières années : au lieu de maximiser le rendement maximal soutenu, elle met maintenant l'accent sur le rendement économique maximal en tenant compte des coûts et des revenus de la pêche. Le présent article décrit un modèle permettant de déterminer le taux de capture et la grandeur de maille optima pour un rendement économique soutenu dans un modèle de Beverton–Holt utilisant plusieurs cohortes. On y compare l'effort et la grandeur de maille optima avec ceux prédits dans une pêche non réglementée. Se limitant surtout à un état d'équilibre, l'analyse tente néanmoins de déterminer la réponse optimale de déséquilibre face à des changements exogènes du recrutement résultant de facteurs environnementaux.

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In fisheries economics there are two quite different types of models of the population dynamics: the "logistic" models focussing on the growth of the total population biomass and the more refined models based on "year-classes" or age cohorts deriving from the work of Beverton and Holt (1957). The optimal dynamics of the logistics or Shaeffer model (Shaeffer 1954) have been well described in Clark (1973) and Clark and Munro (1975). This analysis has gone a long way to highlight the problem of overfishing and the consequent danger of stock extinction in many unregulated fisheries. To discuss optimal mesh regulation as well as overall quota limitation, however, requires the employment of a Beverton–Holt model.

Because of the complexity of distinguishing many age-classes, little work has been done to describe optimal economic exploitation in a multicohort fishery. The pioneering work of Clark et al. (1973) focussed largely on the behavior of a single year-class. Subsequent work on the optimal harvesting of structured populations has been done by Getz (1979) and Botsford (1981). My analysis differs from that of these authors in the assumptions here of increasing cost to fishing effort (due to crowding or differing productivities of fishing grounds) and of increases in fishing costs resulting from stock decline. These assumptions rule out the pulse fishing allowed by Clark's analysis (which assumes an exogenous maximum fishing effort) and, by determining effort, allows a comparison of optimal and competitive industry effort levels.

The analysis proceeds as follows: first, the multicohort population model is described; then steady-state optimal fishing effort and minimum age of capture are determined; an attempt is then made to describe the optimal dynamic response of fishing mortality (effort) and minimum age of capture to changes in recruitment resulting from environmental factors exogenous to the model; the final section compares optimal and competitive industry effort levels, although this analysis is limited to comparisons of alternative steady states.

The Multicohort Population Model

The model I employ is a deterministic model of a single species utilizing the Beverton–Holt assumptions of a constant natural mortality rate and a total catch rate for the fishery proportional to the stock of fish. It is described by an age-specific continuity equation for the change in $x(a, t)$, the number of fish of age a at time t, in terms of the (constant) natural mortality rate, m, and $f(t)$, the controllable fishing mortality:

$$(1)\qquad \frac{\partial x(a,t)}{\partial t} = -\frac{\partial x(a,t)}{\partial a} - [m + f(t)]x(a,t).$$

This has boundary conditions:

$$(2)\qquad x(0,t) = \int_0^D v(x)x(a,t)da,$$
$$x(D,t) = \bar{x}(D), \qquad x(a,0) = x_0(a).$$

Recruitment $x(0, t)$ is assumed to be population dependent with $v(x)$ the fertility function at a given stock age.

For simplicity, I shall assume the function $v(x)$ of the form

$v(x) = v_0 x^{-\eta}$, the negative effect of stock on fertility arising from population pressure. Combining (1) and (2) given an initial age distribution $x(a, 0)$ completely describes the stock to age D as a function of the previous time path of fishing mortality. For simplicity, and without loss of generality, I also assume a fixed maximum lifetime and a fixed stock level $x(D, t) > 0$. This ending stock must be positive if $D < \infty$ but may be as close to zero as desired.

The Beverton–Holt model also allows for the growth in weight of individual fish by means of a function $w(a)$ representing the average weight of a fish of age a. It is assumed that $w(a)$ is increasing, but at a decreasing rate, so $w'(a)/w(a)$ decreases. This is valid for any concave growth curve and also for the standard von Bertalanffy curve $w(a) = w(a - e^{-ka})^3$. The biomass of a single year-class is thus $b(a, t) = w(a)x(a, t)$, and the total biomass is the sum over all ages

$$B(t) = \int_0^D b(a, t)da.$$

Overall biomass growth follows the equation

$$(3) \quad \frac{dB(t)}{dt} = \int_0^D w'(a)x(a, t)da + w(0)x(0) - w(D)x(D) - (m + f)B(t).$$

Optimal Harvesting with Many Age-Classes

The determination of optimal harvesting in a multicohort fishery with constant per-unit costs of effort has been discussed by Getz (1979) and Botsford (1981). The optimal policy in this case will usually be a form of singular control leading to pulse fishing. To better compare optimal with competitive market results the alternative assumption of increasing costs to fishing effort is made here. This may result, for example, from the presence of a scarce factor such as experienced captains and crew, or from crowding of the fishing grounds. The consequence of this assumption is to ensure an interior solution for equilibrium effort. Allowing stock depletion to affect costs as well and assuming fishing mortality, f, proportional to effort results in a cost function specification in terms of f and x of the form $C_1(f)C_2(x)x$ with $C_1'(f) > 0$, $C_1''(f) > 0$, and $C_2'(x) < 0$.

Let $\bar{p}$ represent the (constant) price per pound of fish caught, assumed to be independent of weight. Harvesting revenue from a cohort of age a is thus $\bar{p}w(a)x(a, t)f(t)$. Assuming knife-edge mesh selectivity allows capture of all cohorts above a certain size corresponding to age $\bar{a}$. Total yearly net revenue is thus

$$(4) \quad \pi(t) = \int_{\bar{a}(t)}^D [\bar{p}w(a) - C_1(f)C_2(x)]x(a, t)da.$$

With δ the constant discount rate, the optimal policy for the fishery is to maximize the present value of $\pi(t)$, or the double integral

$$(5) \quad J = \int_0^\infty \int_{\bar{a}}^D e^{-\delta t}[(\bar{p}w(a)f - C_1(f)C_2(x)]xdadt$$

subject to

$$\frac{\partial x}{\partial t} + \frac{\partial x}{\partial a} = -[m + f(t)]x(a, t), \qquad x(D, t) = \bar{x}(D)$$

and

$$x(0, t) = \int_0^D v(x)x(a, t)da.$$

This is a problem in the applied optimal control of distributed parameter systems. Its solution (shown in the Appendix) results in the following necessary first-order conditions for a maximum, where $\lambda(a, t)$ represents the shadow value of the stock in the age a cohort:

$$(6) \quad \int_{\bar{a}}^D [\bar{p}w(a) - C_1'C_2 - \lambda(a, t)]xda = 0$$

$$(7) \quad \frac{\partial \lambda}{\partial t} + \frac{\partial \lambda}{\partial a} = (m + \delta + f)\lambda - [\bar{p}wf - C_1(C_2 + xC_2') + v_0(1 - \eta)\lambda(\bar{a})x^{-\eta}]$$

$$(8) \quad pw(\bar{a}) - AC(\bar{a}) = \lambda(\bar{a}, t)\left[1 - \frac{\partial x(\bar{a})/\partial t}{fx}\right]$$

$$(9) \quad \lim_{t \to \infty} e^{-\delta t}\lambda(a, t) \geqslant 0, \qquad \lim_{t \to \infty} e^{-\delta t}\lambda(a, t)x(a, t) = 0.$$

Equation (6) represents the condition for optimal fishing mortality or the optimal provision of effort in a fishery. Because it is not possible to select fish of a specific age but only those above $\bar{a}$, ($f(t)$ is not a function of age), maximization with respect to f requires marginal revenue from effort equal to full marginal cost over all fishable ages instead of for each age separately. The optimal interrelationship between the value of the fish stock at different ages is defined by equation (7), which, along the path to the optimal steady-state harvest, is a differential equation in both age and time. The stock shadow price of fish of a given age optimally increases at rate ($m + \delta + f$) reflecting discounting and increasing stock scarcity due to mortality. The effect of a stock reduction on future fertility also affects λ, as reflected in the last term of equation (7), and the negative middle term reflects the reduction in rent from a given cohort resulting from a decline in its stock. Equation (8) determining the optimal minimum harvest age is analogous to the condition described by Gordon (1967) for the determination of the optimal extraction lifetime of a nonrenewable mineral deposit; that extraction should proceed until the net profit on the ore of cutoff grade just equals zero. In this case the cutoff age occurs where the net value of harvesting fish a year younger just equals the reduction in the value of the stock by depleting the younger cohorts. Finally, (9) presents transversality conditions that ensure that $\lambda(a, t)$, the shadow price of the stock of age a fish, converges to a constant distribution as $t \to \infty$.

Proof that the optimal paths of $\bar{a}(t)$, $f(t)$, and $x(a, t)$ converge to steady-state values independent of time is not attempted here, although the transversality conditions (9) would seem to imply it, along with the convexity assumptions about costs. Here, I describe the determination of the constant values of the variables assuming that such a steady-state solution exists. According to (9), when in the limit $t \to \infty$, $\partial\lambda/\partial t \to 0$, and the value of the stock becomes simply a function of age. If this is so, employing (8) allows $\partial\lambda/\partial a$ to be integrated:

$$(10) \quad \lambda(a, f, \bar{a}) = e^{ga}\left\{[\bar{p}w(\bar{a}) - AC(\bar{a})]e^{-g\bar{a}} - \int_{\bar{a}}^a e^{-gs}\{f\bar{p}w(s) - C_1(C_2 + xC_2') + v_0(1 - \eta)[\bar{p}w(\bar{a}) - AC(\bar{a})]x^{-\eta}\}ds\right.$$

with $g = \delta + m + f$. The stock shadow price is thus a function of f and $\bar{a}$, as well as age.

In steady state the stock also has a time-invariant age distribution. In fact, a steady-state stock equilibrium with my recruitment relation implies a relationship between $\bar{a}$, the minimum catchable age, and equilibrium fishing mortality. Integrating the recruitment equation (2) frm age 0 to $\bar{a}$ and from $\bar{a}$ to D with the assumed fertility function defines

$$(11) \quad x(0) = v_0{}^{1/\eta}\left[\int_0^{\bar{a}} e^{-m(1-\eta)a}da + e^{(1-\eta)f\bar{a}}\int_{\bar{a}}^{D} e^{-(m+f)(1-\eta)a}da\right]^{1/\eta} = \psi(f, \bar{a}).$$

As long as $\eta < 1$, so a larger stock increases total recruitment, then $\partial x(0)/\partial f < 0$ and $\partial x(0)/\partial \bar{a} > 0$. The constant end-stock level $\bar{x}(D) = x(0)e^{f\bar{a}}e^{-(m+f)D}$ then implicitly defines a positive relationship between f and $\bar{a}$. To maintain the stock at steady state, an increase in fishing must be offset by a catch reduction through increase in mesh size.

To determine f and $\bar{a}$, another equation is necessary, and this is provided by substituting $\lambda(\bar{a})$ from (10), which is a function of f and $\bar{a}$ as well as age, into equation (6). Integrating $\int \lambda x$ by parts, (6) becomes

$$(12) \quad \delta \int_{\bar{a}}^{D} (pw(a) - C_2C_1{}')xda = \lambda(D)\bar{x}(D) - \lambda(\bar{a})x(\bar{a}) + \int_{\bar{a}}^{D} e^{-ga}x[\bar{p}wf - C_1(C_2 + xC_2{}') + \bar{v}(1-\eta)\lambda(\bar{a})x^{-\eta}]da$$

an implicit function only of f and $\bar{a}$, along with the cost, discount and natural mortality rates, and other biological parameters. Equations (11) and (12), along with the constant end-stock level, thus determine the optimal fishing mortality rate and minimum age of catch required to maintain the fishery in steady-state equilibrium.

Optimal Response to Fertility Change

In practice, a fishery will almost never be in steady state equilibrium due to environmentally caused exogenous fluctuations in recruitment. These can be represented in the model by exogenous changes in the fertility parameter, v_0. What will be the optimal response to a permanent increase in v_0, starting from a steady-state equilibrium?

Exogenous recruitment change will affect optimal f and $\bar{a}$ gradually in the model, through the change in the stock shadow price, beginning with $\lambda(\bar{a}, t)$, the value of a fish at the minimum age of capture. For given f and $\bar{a}$, $\lambda(\bar{a}, t)$ will be determined from (8) as a function of the percentage change in $x(\bar{a}, t)$ (denoted by $\hat{x}(\bar{a}, t)$), which results from the change in fertility $\bar{a}$ years previously. This is

$$\hat{x}(\bar{a}, t) = e^{-g\bar{a}}\hat{v}_0(t - \bar{a}) + (1 - \eta)^2 v_0(t - \bar{a})e^{-g\bar{a}} \times \int_0^D x(a, t-a)^{-\eta}\partial x/\partial t da.$$

The increase in fertility affects $x(\bar{a}, t)$ initially $\bar{a}$ years after the increase, then subsequently as the result of secondary increases in fertility due to the higher stock. Thus, $\lambda(\bar{a}, t)$ declines over time, and given that $\partial\lambda(a, t)/\partial t$ was zero in steady state, it is clear from (7) that $\lambda(a, t)$ will decline for subsequent stock ages as well. Equation (6) then implies an increase in optimal fishing mortality ($f(t)$) in response to lower $\lambda(a, t)$ values.

If $x(\bar{a}, t)$ were constant in steady state, an increase in fishing mortality would necessarily imply increase in the minimum age of capture to keep the end stock ($\bar{x}(D)$) at a constant level. The optimal change in $\bar{a}$, however, cannot be determined a priori because $x(\bar{a}, t)$ changes as well. From the stock constraint

$$\frac{d\bar{a}}{dt} = \left\{\int_{\bar{a}}^{D} \frac{\partial f}{\partial t}[t - (D - a)]da - \hat{x}(\bar{a}, t)\right\} \Big/ f[t - (D - \bar{a})]$$

which is ambiguous in sign.

Comparison of Competitive and Optimal Fishing

In contrast with the sustained, rent-maximizing program of optimal fishing, an unregulated fishery will differ in two respects. The common-property nature of the stock means that an individual fisherman can hold no confidence that the resource will remain intact, due to the danger that a competitor will harvest it. The fisherman must therefore ignore the stock constraint, or equivalently, as Clark (1973) has observed, behave as if the discount rate were infinite. Potential future revenues must be discounted to zero, since they cannot be realized in a competitive situation. The second characteristic of the free-access fishery is Gordon's (1954) classic proposition that zero net profits or "economic rent" will be derived by the competiting fishermen in the long run as long as entry to the fishery is unrestricted.

The competitive fisherman will still consider all age-classes present in the current stock but will ignore recruitment and the progress through the different age cohorts, simply maximizing profits in each year (equation (4)) with $x(a, t)$ exogenous. This results in conditions (6) and (8) with the stock shadow price removed. From (6), the fisherman will set the fishing mortality rate at the level where the total value of all year-classes just equals the current marginal cost of fishing all classes. Similarly, the fisherman will not exploit all age-classes but only up to the point where the fish have no current economic value, or $\bar{p}w(\bar{a})f = C_1C_2$. As entry will squeeze the rent from a competitive fishery (so $\pi(t) = 0$), equations (4) and (6) together imply effort (and fishing mortality) determined at the minimum of the average cost of effort, where $C_1'(f) = C_1(f)/f$, assuming that a unique minimum exists. This occurs through the increase in cost occasioned by an overused fish stock. For a given $x(\bar{a})$ the minimum age of capture is determined from $\bar{p}w(\bar{a})/C_2[x(\bar{a})] = C_1(f)/f$. It is clear that this implies overfishing of each age-class relative to the optimum and the setting of a minimum age of catch lower than optimal.

Whether an unregulated fishery succeeds in the destruction of the resource depends on the strength of the effect of a stock reduction on the cost of effort ($C_2(x)$). The equilibrium f and $\bar{a}$ determined above are without reference to any long-run stock equilibrium. The change in the total stock of all ages is

$$(13) \quad dX(t)/dt = x(0) - x(D) - (m + f)X(t)$$

which will be negative if f is large enough. However, if $x(a)$ declines and $C_2(x)$ rises rapidly, this will reduce profit-maximizing fishing mortality. Two cases are illustrated in Fig. 1: one in which $C_2{}'(x)$ is large enough to allow long-run equilibrium where $dX/dt = 0$ and one where $C_2{}'(x) = 0$ so $dX/dt < 0$ indefinitely. In comparison with the optimum equilibrium age distribution of the stock, $x(a)^o$, the unregulated fishery may produce an equilibrium stock at a lower level ($x(a)^{u_1}$) or a nonsustainable disequilibrium situation with the age distribution continually shifting inward over time.

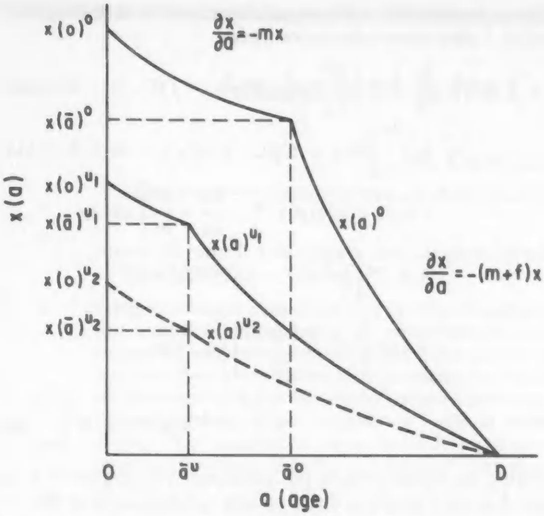

FIG. 1. Typical age distributions of the stock in optimal (*o*) and unregulated (u_1 or u_2) industry fishing.

A Numerical Example

As an example of optimal versus unregulated fishing I employ the parameters of the North Sea plaice (*Pleuronectes platessa*) fishery derived by Beverton and Holt. For this species, the natural growth parameters are as follows: natural mortality rate $= m - 0.10$ and weight curve $w(a) = \bar{w}[1 - e^{-k(a-\pi)}]^3$, where $\bar{w} = 2.867$ kg, $\tau = -0.815$ yr, and $k = 0.095$. The landed price per kilogram was taken as \$0.184 (the value in 1976 from Statistics Canada, *Fishery Statistics* (1979)), the discount rate was assumed to be 10%, and an arbitrary maximum lifetime of $D = 30$ was fixed. For simplicity, the effect of stock changes on fishing costs was ignored and $C_2(x)$ was assumed to equal 1.0. The relationship of fishing mortality and cost was assumed to be quadratic, with $C_1(f) = cf^2/2$ and $\partial C_1/\partial f = cf$. As the cost parameter, c, was not known, it was initially simply set equal to unity. Recruitment was assumed to be exogenous and the stock constrained to be in steady state. A simple iterative process programed in Fortran was then used to solve for the optimal values of f and $\bar{a}$, which, with the parameters above, were found to be $f = 0.1145$ and $\bar{a} = 9.937$, with a cutoff catch weight of $w(\bar{a}) = 0.751$ kg.

These optimal values depend, of course, on all the parameters of the model. Two of the main parameters of interest are the discount rate and the cost parameter. The effect of increasing the discount rate as raising the level of fishing mortality and simultaneously raising $\bar{a}$ to preserve steady-state stock equilibrium is shown in Table 1. The unregulated fishery is represented by a value of $\delta = \infty$ in the table. Unlike Fig. 1, $\bar{a}$ is larger here than for the optimally regulated fishery because $x(0)$ is constrained to be equal in both cases and the stock to be in steady state. The expected negative relationship between optimal fishing mortality and the cost of effort is illustrated in Table 2.

TABLE 1. Simulated relationship of the discount rate with steady-state optimal fishing mortality and the minimum age of catch.

Discount rate	Optimal values of		
$(\delta)^a$	f	$\bar{a}$ (yr)	$w(\bar{a})$ (kg)
0.075	0.1062	8.359	0.5643
0.10	0.1145	9.937	0.7513
0.15	0.1272	11.934	0.9925
∞	0.3662	19.970	1.834

[a] $C = 1.0$; $\delta = \infty$ represents an unregulated fishery.

TABLE 2. Simulated effect of changes in the cost of fishing effort on optimal fishing mortality and the minimum age of catch.

Cost of effort parameter $(C)^a$	Optimal values of f	$\bar{a}$ (yr)	$w(\bar{a})$ (kg)
0.5	0.1292	12.218	1.0264
1.0	0.1145	9.937	0.7513
1.5	0.1062	8.351	0.5633

[a] $\delta = 0.10$.

Discussion

Because of the complexity of the model, the economic determination of optimal catch rates and mesh size in a Beverton–Holt fishery has typically been limited to a single cohort, or equivalently, to the model in biological steady state. By employing the technique of parametric optimal control, I have outlined the optimal dynamics over all fishable cohorts. When the stock is restricted so it is in long-run steady state, a simple simulation illustrates the relationship of the optimal fishing mortality rate and minimum mesh size with the social rate of discount and other parameters of the model. When the stock is not in steady-state equilibrium the analysis is more difficult, but it is possible to show the optimal direction of change in the policy variables in response to changes in recruitment. An unregulated, open access fishery (where the discount rate is effectively infinite) is shown to set the fishing mortality rate too high and minimum age of catch too low, relative to the social optimum.

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Appendix

Adjoining the constraints to the present value performance integral J (equation (5)) yields the following augmented integral:

$$J' = \int_0^\infty \int_{\bar{a}}^D e^{-\delta t}\Big\{\bar{p}w(a)f - C_1(f)C_2(x)]x - \lambda\left[\frac{\partial x}{\partial t} + \frac{\partial x}{\partial a} + (m+f)x\right]\Big\}\, dadt.$$

Integration of

$$\int_{\bar{a}}^D \lambda \frac{\partial x}{\partial a} da = \lambda x \Big|_{\bar{a}}^D - \int_{\bar{a}}^D x \frac{\partial \lambda}{\partial a} da$$

by parts with

$$x(D) = 0 \text{ and } x(\bar{a}, t) = x(0, t)e^{-m\bar{a}} = e^{-m\bar{a}} \int_0^D xv(x)da$$

yields

$$J' = \int_0^\infty \Big\{\Big(\int_{\bar{a}}^D e^{-\delta t}\Big[\bar{p}w(a)f - C_1(f)C_2(x) - \lambda\left(\frac{\partial x}{\partial t} + m + f\right) + \frac{\partial \lambda}{\partial a} + \lambda(\bar{a})v(x)\Big] xda + \lambda(\bar{a})e^{-m\bar{a}} \int_0^{\bar{a}} v(x)xdae^{-\delta t}\Big\}\, dt.$$

The variation of J' with respect to a variation in fishing mortality f after some substitution yields

$$\begin{aligned}\Delta J' = \int_0^\infty e^{-\delta t}\Big(\Big(\int_{\bar{a}}^D [\bar{p}w(a) - C_1'(f)C_2(x) - \lambda]xda\Delta f \\ + \int_{\bar{a}}^D \Big[\bar{p}wf - C_1(C_2 + xC_2') - (m + \delta + f)\lambda \\ - v_0(1-\eta)\lambda(\bar{a})x^{-\eta} + \frac{\partial \lambda}{\partial a} + \frac{\partial \lambda}{\partial t}\Big] xda\Delta x \\ + e^{-m\bar{a}}\Big\{pw(\bar{a})f - C_1(f)C_2[x(\bar{a})] \\ - \lambda(\bar{a})\left[1 - \frac{\partial x(\bar{a})/\partial t}{fx}\right]\Big\}\Delta\bar{a}\Big)\, dt \\ + \int_{\bar{a}}^D e^{-\delta t}\lambda(a, t)x(a, t)\Big|_{t=0}^{t=\infty} da.\end{aligned}$$

Setting the coefficients of the variations with respect to f, x, and $\bar{a}$ to zero results in the necessary conditions (6) to (9).

Analysis of Echo Counting Data: A Model

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Kieser, R., and T. J. Mulligan. 1984. Analysis of echo counting data: a model. Can. J. Fish. Aquat. Sci. 41: 451–458.

Echo counting is a standard tool for the estimation of fish density and hence population. Density estimates are readily obtained using an uncalibrated, stable echo sounder with accurate time-varied gain. We extended a standard nonstochastic echo pulse counting model to include the analysis of single fish echo traces as they are obtained from the sounder chart recorder. The trace counting analysis depends strongly on the overlap in the insonified volumes between successive sounder transmissions. An equation for the nonoverlapping volume is given in terms of range, distance between transmissions, and effective beam angles. The near and far range limits of the model are given and the errors associated with the commonly used approximations to the correct model are shown. A generalization of the model considers the analysis of data from a tilted transducer. A sensitivity analysis gives a qualitative indication of the model's performance for echoes from fish with a realistic target strength distribution. Single fish echo returns are analyzed by the echo pulse and trace counting methods to illustrate the model.

Le comptage des échos est une méthode standard pour estimer la densité des poissons et, partant, celle de l'effectif d'une population. Si l'on utilise un écho-sondeur stable, non calibré, avec gain à variation temporelle, on obtient facilement une estimation de la densité. Nous avons élargi un modèle de comptage d'impulsions sonore non stochastique standard de façon à inclure l'analyse de traces d'échos d'un seul poisson, telles qu'elles apparaissent sur la bande enregistreuse d'un écho-sondeur. L'analyse des comptages de traces dépend beaucoup du chevauchement des volumes intensifiés entre les transmissions successives du sondeur. Nous présentons une équation pour volume sans chevauchement, en termes de portée, de distance entre transmissions et d'angle effectif du faisceau. Les limites de portée, à proximité et à distance, du modèle sont données, de même que les erreurs associées aux approches communément adoptées du modèle correct. Dans une généralisation du modèle, nous évaluons l'analyse des données recueillies à l'aide d'un transducteur incliné. Une analyse de sensibilité donne une appréciation qualitative de la performance du modèle pour des échos de poissons avec une repartition adéquate de l'index de réflexion des cibles. Enfin, le modèle est illustré à l'aide d'une analyse de retours d'échos de poissons uniques, par des comptages d'impulsions d'échos et de traces.

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Echo counting is a standard method for the assessment of fish stocks. Unlike echo integration, which requires a hydroacoustic system that is calibrated against an absolute standard, echo counting can produce an absolute population estimate with an uncalibrated but stable echo sounder. If the sounder has an accurate time-varied gain (TVG) of $40 \log R + 2\alpha R$, analysis of the return echoes from each acoustic transmission (ping) is straightforward. This method of fish density estimation (echo pulse counting) is commonly employed when using a commercially available echo counting system. Analysis is more difficult if all echoes from an individual fish are reckoned as a single count (trace counting) because the adjacent insonified volumes will overlap as the range from the transducer increases. Trace counts are typically obtained from the echosounder chart paper (echogram) by visually counting the characteristic single fish echoes (inverted V shapes). Traditionally, trace counting data are analyzed by an approximate procedure. The total insonified volume is either assumed to be conical, for the case of nonoverlapping insonified volumes, or wedge shaped for highly overlapping volumes (see Fig. 1 and 2). In the transition region of light to moderate overlap, neither model is appropriate. In comparison with traditionally used restricted models (Cushing 1968; Harris 1968; Forbes and Nakken 1972; Clay and Medwin 1977) the present work gives a unified approach to both echo pulse and trace counting. In particular, it presents an extended echo trace counting model that is valid for any degree of overlap in the insonified volume.

The Model

Our nonstochastic echo counting model is based on the following assumptions:

1) The echoes do not overlap.

2) The insonified fish are of uniform size and it is reasonable to assume a single representative target strength (TS).

3) The echo sounder is stable and its gain has been chosen such that noise or echoes from small fish fall below a threshold and are not recorded. Echoes from targets in the side lobes of the acoustic beam also fall below this threshold.

4) The sounder has an accurate $40 \log R + 2\alpha R$ TVG. If an accurate TVG is not available the basic sampling volume will not be conical and the data analysis depends on the particular sounder gain function.

5) The fish do not avoid the survey vessel, and the density of the insonified fish is representative for the survey area.

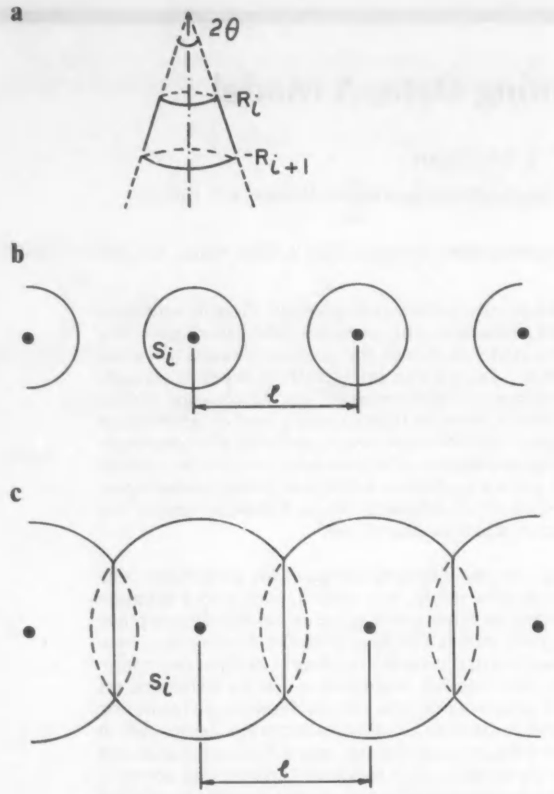

FIG. 1. (a) View of the insonified volume V_i. The range boundaries are given by R_i and R_{i+1}. The angle θ lies in a plane that is defined by the beam axis and the direction of the vessel motion. (b) On-axis view of the insonified area S_i at range R_i. The interping distance l is measured parallel to the vessel motion. The insonified areas do not overlap. (c) The insonified areas overlap; the nonoverlapping areas for trace counting are bounded by the solid lines.

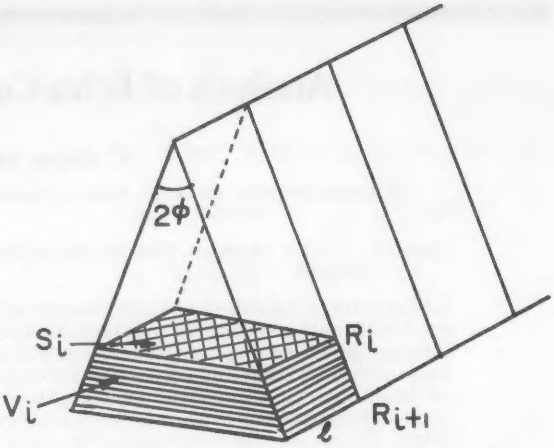

FIG. 2. Wedge-shaped volume for several consecutive pings.

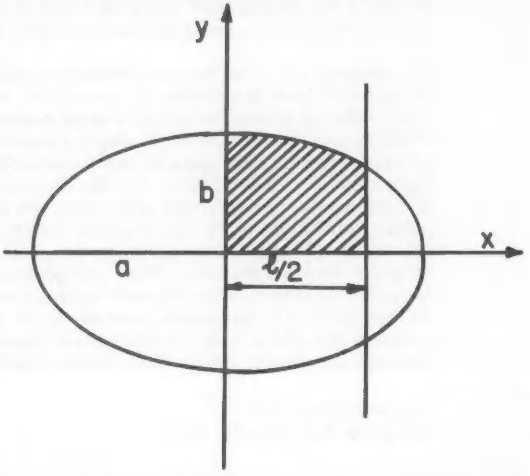

FIG. 3. Elliptical area insonified by a rectangular transducer. The x-axis is parallel to the vessel motion. The shaded area gives one quarter of the nonoverlapping area.

These are the usual, often implicit assumptions for echo counting. The general acceptance of this method may be used as an indication of their validity. The second assumption may be the most critical, as even the same fish will have a widely varying TS depending on small changes in aspect or other parameters. We will show that typical in situ TS distributions are narrow enough for the use with this model.

Under these assumptions all accepted single fish echoes are from targets that lie in a cone subtending an angle of 2θ parallel to and 2φ perpendicular to the motion of the sounding vessel. The angles θ and φ describe a beam of elliptical cross section (Fig. 3) that approximates the volume that is insonified by a rectangular transducer. For a circular piston transducer, $\theta = \phi$. (A circular beam pattern cross section can also be used as an approximate solution for a square transducer.)

The number of echo pulses or traces observed for the depth interval between R_i and R_{i+1} (the ith range slice) is given by

$$(1) \qquad n_i = N \int_{R_i}^{R_{i+1}} \rho(R) S(R) dR$$

where n_i = pulse or trace count for the ith range slice, N = number of pings to be analyzed, R_i = top of ith range slice, R_{i+1} = bottom of ith range slice, $\rho(R)$ = actual fish number volume density (No./m^3), and $S(R)$ = area insonified by a single ping at range R. For pulse counting, this is the area the insonified cone cuts out of a plane that is perpendicular to the acoustic axis and at range R. For trace counting this is the nonoverlapping area defined in Fig. 1c.

Equation (1) can be solved for $\rho(R)$ only if ρ is constant over the range of interest. In practice, an estimate, $\hat{\rho}_i$, of the true fish density, $\rho(R)$, is obtained from the observed n_i:

$$(2) \qquad \hat{\rho}_i = \frac{n_i}{N \int_{R_i}^{R_{i+1}} S(R) dR} = \frac{n_i}{NV_i} .$$

For pulse counting, V_i is the total insonified cone volume, while for trace counting it is the corresponding nonoverlapping volume. If $\rho(R)$ is approximately constant for R_i to R_{i+1} or if $R_{i+1} - R_i \ll R_i$ then $\hat{\rho}_i$ will give a good estimate of the average fish density within the insonified volume. Under these conditions the family of $\hat{\rho}_i$'s represents a reasonable approximation to the actual variation in fish density, ρ, as a function of R.

The density estimate can be expanded to a population estimate P:

$$P = A \sum_i \hat{\rho}_i (D_{i+1} - D_i)$$

$$(3) \qquad P = (A/N) \sum_i \frac{n_i(D_{i+1} - D_i)}{V_i}$$

where $D_i = R_i \sin \gamma$, depth beneath the transducer, γ = angle between surface of the water and the beam axis, A = surface area associated with the sampled water volume. The summation is over all range slices of interest. As usual, it is assumed that the insonified volume V_i has a fish density that is representative of the corresponding volume under the surface area A. A survey area may be sampled by several acoustic transects. Each A then represents a fraction of the total survey area. The total area will be equal to the sum over the A's, and the total population estimate will be equal to the sum over the estimates P.

For the moment a vertical transducer with $\gamma = 90°$ is assumed. The principal parameter that remains to be estimated is the insonified volume V_i.

Echo Pulse Counting

For the case of echo pulse counting the insonified volume is simply the total insonified volume, regardless of overlap. For an elliptical beam the insonified area $S(R)$ at range R_i is

$$(4) \qquad S(R) = \pi R^2 \sin\theta \sin\phi.$$

The range R_i to R_{i+1} delineates the volume V_i:

$$(5) \qquad V_i = (1/3)(R_{i+1}S_{i+1} - R_iS_i).$$

If θ and ϕ are sufficiently small, equations (4) and (5) will be good approximations to the curved surface that actually delineates the range R_i and to the volume V_i bounded between two such surfaces. This approximation when applied to the volume for a circular piston transducer of $\theta = 10°$ gives an answer within 1% of the true curved surface volume (Burington 1973):

$$V = (2/3)\pi(R_{i+1}^3 - R_i^3)(1 - \cos\theta).$$

A similar model was used by Ehrenberg and Lytle (1972), who evaluated the bias that will arise in the echo pulse counting estimate when the fish density increases and the received echoes begin to overlap. A second source of bias (not discussed by these authors) originates when the observed TS's show a large spread.

Echo Trace Counting

In its simplest form, echo trace counting only requires an echo sounder. Traditionally the analysis has used the above model with a cone or wedge shape for the insonified volume (Forbes and Nakken 1972), depending on the degree of overlap between the volumes that are insonified by successive transmissions. We now present a trace counting model that is correct for any degree of overlap.

Trace counting reduces to pulse counting as long as the range R is sufficiently small so that insonified volumes do not overlap between transmissions (Fig. 1). This is true if

$$(6) \qquad \ell \geqslant 2R \sin\theta.$$

The distance between pings, ℓ, is given by the transect length L divided by the number of transmissions N:

$$\ell = L/N.$$

When the range becomes larger the cones begin to overlap and the insonified area $S(R)$ and volume V_i are no longer given by equations (4) and (5).

To derive the expression for the nonoverlapping insonified area, consider the ellipse shown in Fig. 3. The periphery is given by

$$y = (b/a)\sqrt{(a^2 - x^2)}$$

where $a = R \sin\theta$ and $b = R \sin\phi$ are the major and minor half axes. The nonoverlapping insonified area is bounded by $x = \pm\ell/2$ and is given by

$$(7) \qquad S(R) = 4\int_0^{\ell/2} (b/a)\sqrt{(a^2 - x^2)}dx$$

$$= \ell b[\sqrt{1 - (\ell/2a)^2} + (2a/\ell)\sin^{-1}(\ell/2a)]$$

$$= \ell R \sin\phi\, [\sqrt{1 - q(R)^2} + (1/q(R))\sin^{-1} q(R)].$$

In equation (7), $q(R)$ is a convenient, dimensionless quantity defined as the ratio of interping distance divided by the beamwidth in the fore–aft direction at range R:

$$q(R) = \frac{\ell}{2R\sin\theta}.$$

The insonified volume V_i is obtained by integrating $S(R)$ between the range boundaries R_i and R_{i+1}:

$$(8) \qquad V_i = \int_{R_i}^{R_{i+1}} S(R)dR.$$

This yields (Appendix)

$$(9) \qquad V_i = 1/3\ell R_i^2 \sin\phi \left\{ 2\sqrt{1 - q_i^2} + 1/q_i \sin^{-1} q_i - q_i^2 \ln[(1/q_i)(1 + \sqrt{1 - q_i^2})] \right\}_{R_i}^{R_{i+1}}.$$

The first-order expansion of $S(R)$ and V_i in q can be used when the insonified volumes overlap strongly, i.e. $2R\sin\theta \gg \ell$ or equivalently, $q \ll 1$. This approximation gives

$$(10) \qquad S(R) = 2\ell R \sin\phi$$

and

$$(11) \qquad V_i = \ell \sin\phi\,(R_{i+1}^2 - R_i^2)$$

where $S(R)$ is the rectangular area shown in Fig. 2 and V_i gives the wedge-shaped volume shown in the figure between R_i and R_{i+1}. This volume has been traditionally used to analyze echo trace counting data when the insonified volumes overlap strongly.

The volume of equation (9) is compared with the cone model and wedge model volumes in Fig. 4. A thin depth slice has been chosen for this figure; otherwise, typical parameters are assumed. The volume for trace counting is given by the heavy curve. By definition it coincides with the cone volume at ranges where the beams do not overlap. As the range increases it asymptotically approaches the wedge volume.

Effective Beam Angles θ and φ

A typical choice for the effective beam half angles θ and ϕ is shown in Fig. 5. On the average, targets outside these angles

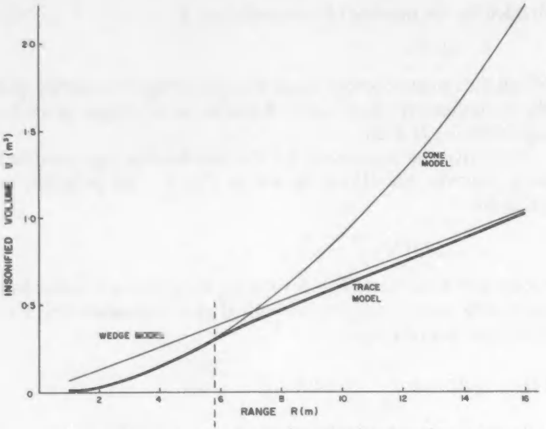

FIG. 4. Volumes used in the echo pulse and trace counting analysis plotted against range. Typical parameters have been used: $\theta = 7.4°$, $\phi = 12.5°$, $\ell = 1.5$ m, $R_{i+1} - R_i = 0.1$ m, $\rho =$ constant.

produce small echoes that fall below the recording threshold and are ignored. It is convenient to define a corresponding beam threshold BT. Generally, BT should lie above the maximum side lobe level but within the steep portion of the main lobe. Thus, no fish are detected in the side lobes and the effective beam angles are least dependent on BT and consequently TS.

The angles θ and ϕ can be estimated from a measurement of the relative echo sounder gain and the transducer beam pattern or an in situ measurement that uses an acoustic target. A knowledge of the absolute systems gain or the fish's TS is not required. For both measurements the dynamic range, DY, of the accepted fish echoes must be known. It is given by

$$\mathrm{DY} = \mathrm{GN}_{\max} - \mathrm{GN}_{\min} = 20 \log \frac{v_{\max}}{v_{\min}}$$

where GN is the relative systems gain. DY and GN are measured in decibels.

If the echo sounder has a calibrated gain control then the dynamic range is readily determined by reducing the gain from the normal operating level, $\mathrm{GN}_{\max}$, to a level, $\mathrm{GN}_{\min}$, where only the strongest echoes are recorded. Alternatively, the echoes can be monitored on an oscilloscope and a "reasonable maximum" echo level, $v_{\max}$, can be estimated as the average voltage from the k_1 largest echoes:

$$v_{\max} = \frac{1}{k_1} \sum_{k=1}^{k_1} v_k.$$

This requirement stems from the fact that the observed fish TS's are not a constant but will have a certain spread. The factor $1/k_1$ should correspond to the fraction of solid angle that is given by the relatively flat central portion of the acoustic beam pattern. Echoes that fall below the threshold voltage, $v_{\min}$, are not counted. For trace counting from the echogram, the voltage threshold for marking the chart paper will determine $v_{\min}$.

DY is used to determine the BT by

$$\mathrm{BT}(\theta) = \mathrm{BT}(\phi) = \mathrm{DY}/2.$$

Only half the dynamic range is used to find BT as the beam pattern is measured one way whereas the sound pulse is both transmitted and received by the transducer. The BT determines the half angles θ and ϕ either graphically as illustrated in Fig. 5 or by the appropriate beam pattern equation.

Alternatively, an acoustic target can be used for the experimental determination of the effective beam angles. The procedure requires careful positioning of the target on the beam axis and at locations parallel and perpendicular to the planned direction of vessel motion (Fig. 6). The target is first placed on axis to determine the maximum echo level or the minimum echo sounder gain at which the echo is just recorded. Then it is raised, moved off axis, and lowered to a depth at which the echo has increased to the minimum echo level that will be recorded. The effective beam half angle then is given by $\tan^{-1}(AB/BC)$. This measurement can be done in the field if the water is extremely calm.

Oblique Transducer Orientation

In some cases a sonar or echosounder with oblique transducer orientation may be desirable. An oblique transducer orientation has several advantages: the sampling volume is relatively close to the surface, it is at a greater distance from the survey vessel, and it is $(\sin \gamma)^{-3}$ larger for a given depth interval. On the other hand, the following serious disadvantages must be considered:

1) The spread in TS increases drastically as fish are insonified in front and side rather than dorsal aspect. If the range in TS is too large it is difficult to define meaningful effective beam angles θ and ϕ.

2) A stable average TS may not exist when the fish are oriented by a current or some other means.

3) The depth resolution will be degraded when fish layers are insonified by an oblique beam. At the boundary of the layer the apparent fish density may lead to an incorrect interpretation of the data.

4) Refraction of the acoustic beam due to temperature and salinity gradients may become significant at large ranges.

In spite of these limitations the use of an oblique acoustic beam may be desirable. An awareness of the likely problems will be advantageous when an oblique acoustic beam is considered.

The geometry for a side-looking transducer is illustrated in Fig. 7. For this case the beam axis is in a plane that is perpendicular to the motion of the vessel and makes an angle γ with respect to the surface. Working in an inlet under favorable sea conditions and with relatively large fish (TS = −27 dB) we found that γ should be of the order of the second minima in the beam pattern or larger to avoid interference from the surface. In our case this resulted in an angle of approximately 20° (Fig. 5).

The analysis of pulse counting data is straightforward for a transducer oriented in any direction: side-looking, forward-looking, or at any intermediate position. The expressions for the density, equations (1) and (2), and those for the insonified area and volume, equations (4) and (5), remain unchanged due to the choice of R, which specifies the range from the transducer rather than the depth. The model also describes the analysis of trace counting data from a side-looking transducer, i.e. the beam axis perpendicular to the vessel motion. As before, equations (1), (2), and (7) through (11) apply unchanged for this case.

In contrast with pulse counting the trace counting model cannot be used for the analysis of data from a transducer that is not oriented in a plane perpendicular to the direction of vessel motion. Due to the vessel motion, successive beam range boundaries S_i are no longer in a common plane and equation (6)

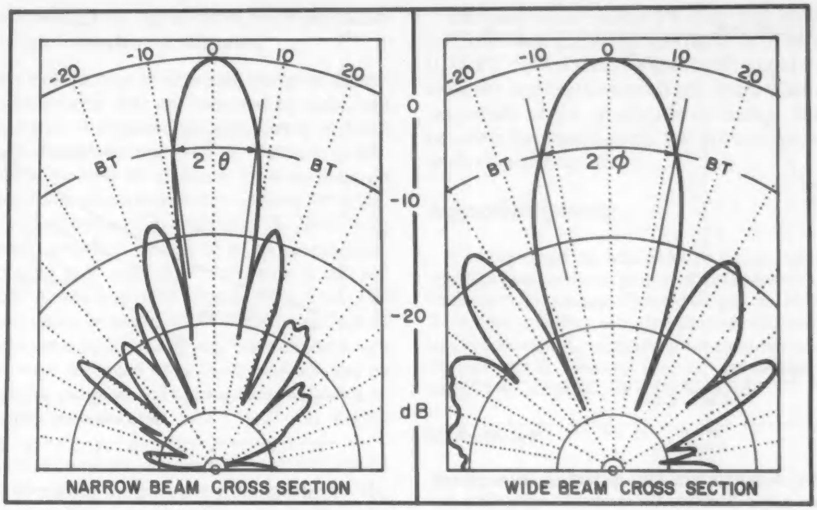

FIG. 5. Polar plots show the measured beam patterns for the rectangular transducer. The distance from the origin gives the relative receive sensitivity in decibels. The beam threshold BT and the effective beam half angles θ and ϕ are indicated.

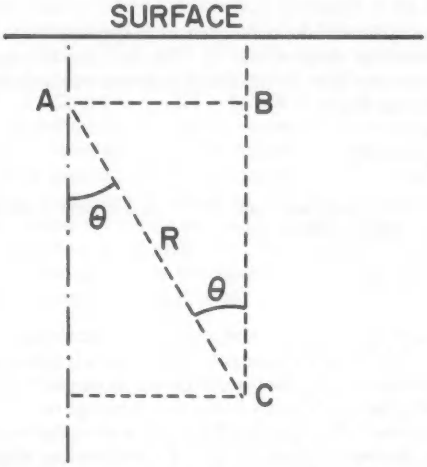

FIG. 6. Effective beam angles can be determined in a simple field measurement. The transducer is located at A, beam axis vertical, and a target is located at C.

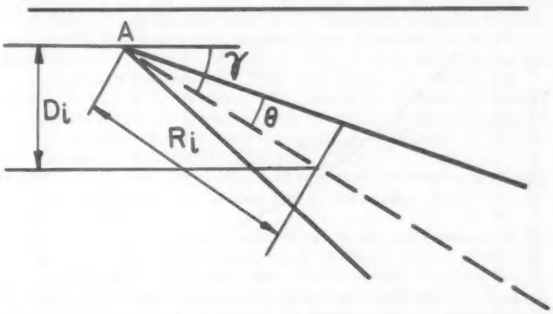

FIG. 7. Geometry of a side-looking acoustic beam. The transducer is located at A; its axis makes an angle γ with the surface. A target at range R_i will be at depth D_i below the transducer.

no longer gives the range at which the insonified areas first overlap. Echo traces from a forward-looking transducer will then appear as a sequence of marks that ascend from the bottom towards the top of the echogram.

Sensitivity Analysis

The error that results from choosing the cone or wedge model for the analysis of trace counting data is illustrated below. A relative population estimate, P/P_0, can be obtained from equations (2) and (3):

$$P/P_0 = V_0/V \approx S_0/S$$

where the zero subscript refers to the trace counting model and the nonsubscripted variables are from either wedge or cone models. When $(R_{i+1} - R_i)/R_i \ll 1$ the approximation in terms of S_0 and S holds. The area ratio depends only on q; consequently, a plot of P/P_0 versus q can be interpreted for any set of parameters, provided the range slice is sufficiently thin. The cone and wedge models are compared with the trace counting model as a function of range in Fig. 8. The parameters are identical to those used in Fig. 4. The trace counting model gives the correct population estimate regardless of range, overlap, etc. The cone and trace models agree for small ranges whereas they diverge for large ranges. Conversely, the wedge model approaches the trace model as the range becomes large. If the trace counting model cannot be used the cone model is optimal for $q > \pi/4$ whereas for smaller q's the wedge model should be used. When this rule is followed the population will never be underestimated by more than 12%. The wedge model will underestimate a population by less than 5% if $q < 0.548$. The figure also indicates that the error increases rapidly when the approximate models are used beyond their optimal range.

We now illustrate the sensitivity of the estimates to errors in the interping distance, ℓ, and beam threshold (target strength) using the population ratio. We define P_0 as the trace counting

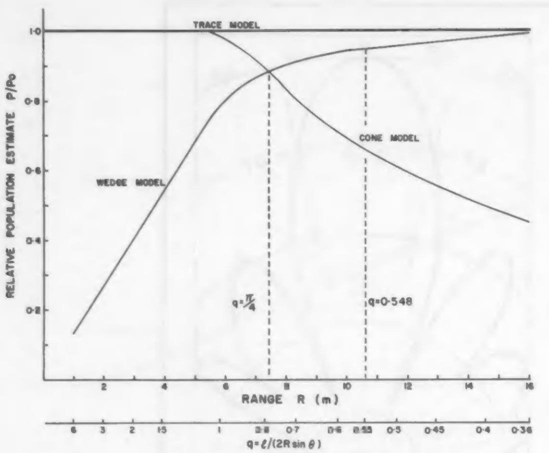

FIG. 8. Relative population estimates P/P_0 plotted against range and $q = \ell/(2R \sin \theta)$. The graph illustrates the errors that arise when the incorrect model is used for the analysis of echo trace counting data. The parameters from Fig. 4 are used.

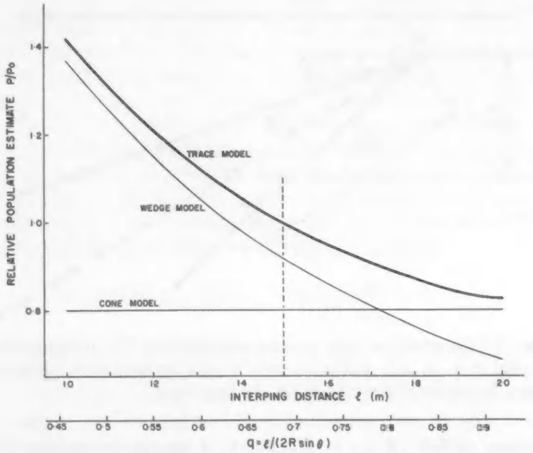

FIG. 9. Relative population estimate as a function of interping distance. P_0 is evaluated for $\ell = 1.5$ m. In addition to the previously used parameters, an 8.5 m range is assumed.

population estimate for a typical set of parameters. In Fig. 9 the relative population estimates are plotted against a range of interping distances that is centered at 1.5 m. A thin range slice at 8.5 m and constant fish density are assumed. The heavy curve is based on the trace counting model and shows relative estimates ranging from 1.42 to 0.823 for interping distances of 1–2 m.

The sensitivity of the population estimates to variations in BT centered at −10 dB and varying from −15 to −5 dB is shown in Fig. 10. For the transducer beam patterns that we used (Fig. 5) this threshold corresponds to a range in effective beam angles θ = 8.6–5.5° and ϕ = 16.1–8.5°. Over this range the relative population estimate varies from 0.737 to 1.56. In practice the uncertainty in the BT is due to variations in TS; thus, Fig. 10 includes a ΔTS scale that gives the change in TS. This scale has a range of 20 dB, twice that for BT.

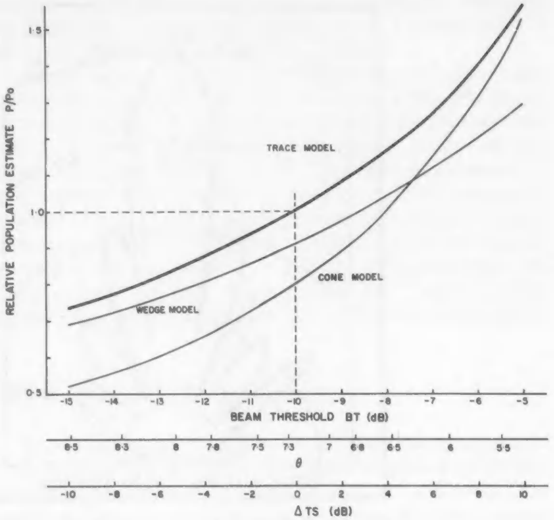

FIG. 10. Relative population estimate as a function of beam threshold. P_0 is evaluated at BT = −10 dB. The parameters from Fig. 9 are used.

Although the model is nonstochastic these figures afford some appreciation of the consequences of ignoring the variance in the interping distance and TS. For the sake of argument assume that the plot of relative population versus interping distance, ℓ, is linear:

$$\frac{P_i}{P_0} = k\ell_i$$

where k is an appropriate constant. From the usual definition of the mean value it follows that

$$\frac{\bar{P}}{P_0} = k\bar{\ell}$$

independent of variance or skewness in the ℓ distribution. Thus, the model will provide a good population estimate as long as the correct mean values for the parameters are chosen and as long as Fig. 9 and 10 are sufficiently linear over the range of ℓ and TS.

In most cases variations in TS will be the dominant source of variance. Recent publications give TS distributions that have been measured in situ on fish that consisted of a single size-class. A summary of the measured TS's and their full width at half maximum (FWHM) is given in Table 1. These values have been read from published figures and must be considered approximate. The significance of the indicated FWHM can be appreciated when it is compared with the 20 dB range of the ΔTS axis (Fig. 10). It is interesting to note that the wedge, trace, and cone models show decreasing linearity in this figure.

TABLE 1. In situ target strength (TS) measurements and the full width at half maximum (FWHM).

TS	FWHM	Units	Species	Size (cm)	Reference
−52	6	dB/fish	Smelt	8	Lindem 1982[a]
−48	6	dB/fish	Smelt	14	Lindem 1982[a]
−28.9	4	dB/kg	Sprat	12.6	Robinson 1982[a]
−32	12	dB/fish	Salmon	—	Ehrenberg 1982[a]

[a] Cited with permission from the author.

Example Demonstrating the Agreement between Echo Pulse and Trace Counting Estimates

The following example serves to illustrate the good agreement obtained when the same data are analyzed by pulse and trace counting techniques. A repeated acoustic survey of Long Lake near Smith Inlet, British Columbia (T. J. Mulligan and R. Kieser, unpubl. data), yielded 64 transects of soundings on adult sockeye salmon (*Oncorhynchus nerka*). During the night, single fish targets were distributed at a depth of 5–20 m, with the majority occurring between 10 and 15 m. A rectangular, ceramic transducer with 8.5° and 13.2° full beam width between the half power points was used to collect the data. The transducer beam axis made an angle of 90° with the surface of the water. The echoes were processed by a receiver channel with 40 log *R* TVG and were recorded on a chart recorder and on analog tape. A special purpose A/D converter was used to transcribe the analog recording to ½-in. (1.27 cm) digital magnetic tape.

The receiver gain was chosen to accommodate all but a small fraction of the largest echoes that saturated the amplifier. The voltage threshold was selected to eliminate echoes from the juvenile salmon and threespine stickleback (*Gasterosteus aculeatus*) populations that occurred at the same depth. The mean weight of adult salmon was 2.9 ± 0.5 kg; that for the small fish was two orders of magnitude lower. A histogram of the accepted single fish peak voltages was used to estimate the dynamic range, yielding DY = 20.8 ± 1.6 dB. From the beam pattern (Fig. 5) the corresponding effective half angles were chosen as $\theta = 7.4 \pm 0.2°$ and $\phi = 12.5 \pm 0.7°$. With an inter-ping distance of 1.32 m the insonified volume overlaps by approximately 30% in the 10–15 m range. Thus, the volume correction between the echo pulse counting and trace counting models is significant. The echo trace count was done by four people from the echograms whereas the pulse count was obtained from a computer-based analysis of the digital tapes. The respective models were used to obtain population estimates PT (trace counting) and PP (pulse counting) for each transect.

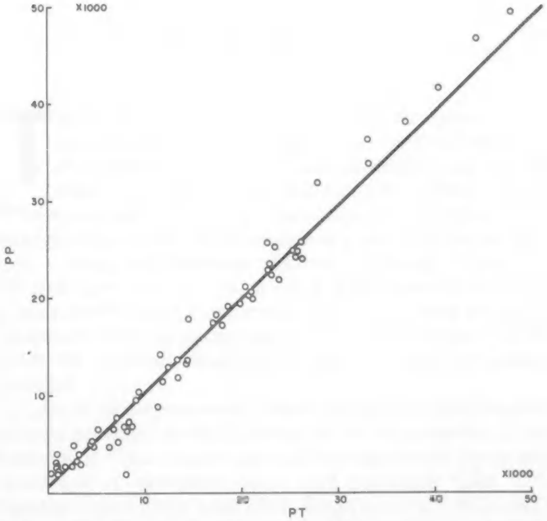

FIG. 11. Pulse counting estimates plotted versus trace counting estimates. Good agreement between the results from the two analyses is evident.

The plot of PP versus PT (Fig. 11) shows the two sets of estimates with a least squares linear fit of PP on PT giving PP = 0.977PT + 94.4 (correlation coefficient of 0.993). The slope is close to 1 and the intercept is negligible when compared with the magnitude of the population estimates. Thus, the agreement between the results from the echo pulse and trace counting analysis was excellent.

Acknowledgments

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Appendix

The nonoverlapping portion of the insonified volume is given by equation (8):

$$V_i = \int_{R_i}^{R_{i+1}} S(R)dR.$$

With the expression for the nonoverlapping area $S(R)$ given by equation (7), we now have

$$V_i = \int_{R_i}^{R_{i+1}} \ell R \sin\phi \, (\sqrt{1-q^2} + (1/q)\sin^{-1} q)dR.$$

A change of integration variable from R to q yields (for the indefinite integral)

$$\begin{aligned} V &= -\frac{\ell^3 \sin\phi}{4\sin^2\theta}\int [q^{-3}\sqrt{1-q^2} + q^{-4}\sin^{-1} q]dq \\ &= -\frac{\ell^3 \sin\phi}{4\sin^2\theta}\left\{\int q^{-3}\sqrt{1-q^2}dq - \frac{q^{-3}}{3}\sin^{-1} q \right. \\ &\qquad \left. + \frac{1}{3}\int \frac{q^{-3}}{\sqrt{(1-q^2)}}\,dq\right\} \end{aligned}$$

$$= \frac{\ell^3 \sin \phi}{4 \sin^2 \theta} \left\{ \frac{q^{-3}}{3} \sin^{-1} q - \frac{1}{3} \int \left[3q^{-3} \sqrt{1 - q^2} + \frac{q^{-3}}{\sqrt{(1 - q^2)}} \right] dq \right\}.$$

These integrals have been tabulated (Burington 1973) and yield for V_i

$$V_i = \frac{\ell^3 \sin \phi}{12 q^2 \sin^2 \theta} \left\{ 2 \sqrt{1 - q^2} + \frac{1}{q} \sin^{-1} q - q^2 \ln \left[\frac{1}{q} \left(1 + \sqrt{1 - q^2} \right) \right] \right\}_{R_i}^{R_{i+1}}$$

$$V_i = \frac{1}{3} \ell R^2 \sin \phi \left\{ 2 \sqrt{1 - q^2} + \frac{1}{q} \sin^{-1} q - q^2 \ln \left[\frac{1}{q} \left(1 + \sqrt{1 - q^2} \right) \right] \right\}_{R_i}^{R_{i+1}}$$

Geochemical Pathways and Biological Uptake of Radium in Small Canadian Shield Lakes

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Hesslein, R. H., and E. Slavicek. 1984. Geochemical pathways and biological uptake of radium in small Canadian Shield Lakes. Can. J. Fish. Aquat. Sci. 41: 459–468.

The sediment–water interactions and biological uptake of ^{226}Ra are described for four small Canadian Shield lakes at the Experimental Lakes Area, Kenora, Ont. A single addition of ^{226}Ra was made to each lake between 1970 and 1976. Approximately 90% of the added ^{226}Ra initially sorbed to the sediments. Outflow from the lakes showed losses of only 5–11% ^{226}Ra per year. Models are proposed for adsorption and outflow of ^{226}Ra from lakes. Biological uptake and long-term ^{226}Ra concentrations were measured in three species of macrophytes, crayfish, and five species of fish. Bioaccumulation ranged from 1100 to 5000 in macrophytes, 750 in crayfish, from 30 to 80 in large adult lake trout (*Salvelinus namaycush*), white sucker (*Catostomus commersoni*), and lake whitefish (*Coregonus clupeaformis*), and from 230 to 1200 in fathead minnows (*Pimephales promelas*), pearl dace (*Semotilus margarita*), and northern redbelly dace (*Chrosomus eos*). The concept of Ra/Ca ratio in organisms versus water and food is used to explain the differences in bioaccumulation. ^{226}Ra is discriminated against versus calcium by fish but favored by macrophytes and crayfish.

On trouvera dans l'article qui suit une description des interactions sédiment–eau et de la captation biologique du ^{226}Ra dans trois petits lacs du Bouclier canadien dans la Région des lacs expérimentaux, à Kenora (Ont.). Entre 1970 et 1976, chaque lac a reçu une dose unique de ^{226}Ra. Environ 90% de cet élément a été sorbé au sédiment. Les émissaires des lacs indiquèrent des pertes de seulement 5–11% de ^{226}Ra par année. Nous proposons des modèles pour l'adsorption et la décharge de ^{226}Ra des lacs. Nous avons mesuré l'absorption biologique et les concentrations de ^{226}Ra à long terme chez trois espèces de macrophytes, dans des écrevisses et cinq espèces de poissons. La bioaccumulation dans les macrophytes était comprise entre 1100 et 5000, dans les écrevisses 750, et entre 30 et 80 chez le touladi (*Salvelinus namaycush*), le meunier noir (*Catostomus commersoni*) et le grand corégone (*Coregonus clupeaformis*) adultes de grande taille, d'une part, et 230–1200 chez le tête-de-boule (*Pimephales promelas*), le mulet perlé (*Semotilus margarita*) et le ventre rouge du nord (*Chrosomus eos*). Nous nous basons sur le concept du rapport Ra/Ca des organismes versus eau et nourriture pour expliquer les différences de bioaccumulation. Les poissons font une distinction en faveur du calcium versus le ^{226}Ra, alors que c'est l'inverse pour les macrophytes et l'écrevisse.

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The behavior of ^{226}Ra in natural aquatic systems is of considerable interest because of its high radiotoxicity, its potential release from uranium mining and nuclear waste materials, and its long half-life (1600 yr). While ^{226}Ra is a naturally occurring radioisotope, its concentrations in biological and chemical components of aquatic systems are very low, making measurements difficult and costly. Typically, 50 kg of water, 10 kg of fish, or 1 kg of plant material is required to measure ^{226}Ra at natural levels even with the most advanced equipment. While some aquatic systems with elevated ^{226}Ra do exist, the amounts, distribution, and dynamics are poorly recorded.

Lakes in the Experimental Lakes Area (ELA) in northwestern Ontario provided an ideal setting for an examination of the behavior of ^{226}Ra. Four of these lakes (morphometric maps and water quality parameters have been published: Lake 224, Hesslein et al. 1980b; Lake 226S, Torgersen et al. 1982; Lakes 227 and 261, Brunskill and Schindler 1971) had known amounts of ^{226}Ra added during the period 1970–78 (Table 1), primarily for the purpose of studying gas exchange with ^{222}Rn, the gaseous daughter product of ^{226}Ra. In the case of the earlier additions, L227 and L261, limited data are available on the change in concentration with time after addition. Emerson et al. (1973) showed ^{226}Ra in water to be only 25% of the initial concentration in L227 1 mo after addition. After 2 mo the concentration was approximately 5% of the initial concentrations. Throughout the 2 mo, the gaseous daughter ^{222}Rn was a higher proportion (30–50%) of the initial concentration because it was escaping from the sediments in contact with the surface waters. The ^{226}Ra had adsorbed to the periphyton that coated the rocks and sediment surfaces. The adsorption occurred whether the periphyton was alive or dead and was independent of the photosynthetic rate (Emerson and Hesslein 1973). Although the deposition of ^{226}Ra was initially only on the epilimnetic sediments as evidenced by ^{222}Rn distributions (Emerson et al. 1973), later work showed that the ^{226}Ra was redistributed throughout the lake (Emerson 1974). In L261, $BaCl_2$ was added with the ^{226}Ra at an atomic ratio of 1 million to 1 in an attempt to

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Table 1. Summary of ^{226}Ra additions to ELA lakes.

Lake	Date	Amount (mBq)	Initial concn. (mBq/L)	1981 surface concn. (mBq/L)	Init. areal concn. (mBq/cm^2)
224	25 July 1976	370	123	8.5	143
226	20 July 1978	185	377	10	238
227	27 July 1970	350	1680	17	740
261	1 Aug. 1971	330	330	15.9	600

inhibit radium adsorption to sediments (Emerson 1974). This did not have the desired effect and few data for ^{226}Ra are available prior to this report.

Radium additions to L224 and L226S were monitored more closely. In L224, ^{226}Ra was added in conjunction with ^{14}C (Hesslein et al. 1980a) and gamma-emitting metals (Hesslein et al. 1980b). ^{226}Ra was lost from the surface water in a roughly exponential manner with a half-time for removal of 61 d. In L226S, ^{226}Ra was added along with tritium (Torgersen et al. 1982). The half-time for removal was about 20 d.

Although the radium addition experiments were not designed for the purposes of this work we have been able to analyze a fairly good temporal and spatial distribution of samples. This was accomplished by analyzing preserved biological and sediment samples from the earlier metal tracer experiments (Hesslein et al. 1980b) as well as collections made for this work in 1981–82. The analyses of the preserved samples along with the published gas exchange data has allowed some interpretation of the short-term uptake rates.

Throughout this work, ^{226}Ra is treated as a stable element because its radioactive decay is too slow to be of importance on the time scale of these investigations.

Methods

Water samples were taken by Van Dorn bottle at the station of maximum depth. The bottle integrates over 0.5 m. Samples were placed in acid-washed bottles and acidified to pH 1 with hydrochloric acid.

Sediment cores were taken by divers in shallow water or with a gravity corer (K-B) in deeper water. Acrylic tubes of 5-cm diameter were used. Cores were extruded and sliced into 1-cm sections on return to the laboratory. Slices were placed in Whirl-Pak® plastic bags and refrigerated. Some older samples had been oven-dried and stored in that state.

Fish were taken by gill net, trap net, or in a few cases by hook and line. Crayfish from L224 were collected in minnow traps, modified to have larger entranceways than usual. All samples were frozen within hours of capture and transported to the laboratory.

Macrophytes were collected in shallow water by wading and uprooting them. Samples were washed to remove settled detrital material and frozen.

Dried (110°C) biological samples were digested in glass beakers by the slow addition of concentrated nitric acid. The sample was then placed on a hot plate in a fumehood and heated until acid fuming ceased. This procedure was repeated as necessary until only a small residue remained. This residue was then fired at 450°C for 6–10 h in a muffle furnace to produce a white ash. Standard additions showed no measurable loss of ^{226}Ra during ashing. The ash was brought into solution in 20 mL of concentrated HCl and then diluted up to 100 mL with distilled water. In the case of the macrophytes, muffling was omitted because little residue remained after acid digestion.

Dried core samples were ground to a fine powder and suspended in 100 mL of 0.1 mol/L HCl. Fresh core samples were brought up to 100 mL and adjusted to 0.1 mol/L HCl. Acid at 0.1 mol/L does not dissolve the sediments but is used to prevent sorption of ^{226}Ra to the walls of the bottle.

Radium was measured by the radon emanation method (Broecker 1965; Mathieu 1977) in which ^{222}Rn, the radioactive daughter of ^{226}Ra, is flushed from the sample and collected. The alpha emissions of ^{222}Rn as well as its daughters ^{218}Po and ^{214}Pb are measured by alpha scintillation counting. This method has a number of advantages. The background counts are very low (0.5 cpm) and the efficiency is high (70–95%). The sensitivity is good because there are 3 emissions per ^{226}Ra decay. The sample ^{226}Ra can be redetermined after allowing ^{222}Rn to accumulate again and almost any sample size can be used. We commonly used from 50 mL to 50 L.

Corrections were made for the time of accumulation of ^{222}Rn and decay while in the counting cell. Cell background (0.2–1.0 cpm) and bottle plus reagent blank (7–18 mBq depending on the vessel) were subtracted. Standardization was done against solutions of known ^{226}Ra concentrations (NBS traceable). Precision of the method was better than ±3% at 10 times the detection limit and degraded to >100% as the detection limit was reached (~18 mBq/sample).

Calcium was measured by atomic absorption spectrophotometry as outlined by Stainton et al. (1974).

Results

Geochemistry

Natural levels of ^{226}Ra in water were measured in three unaltered lakes in the ELA (L239, L302, L223). The values ranged from 0.4 to 3.0 mBq/L in surface waters. The 1980–81 surface values in the spiked lakes exceeded this by an order of magnitude (Table 1). By dividing the amount of ^{226}Ra added to the lakes by the lake volumes (Table 1), one can calculate the mean concentrations if all the ^{226}Ra had stayed in solution. Comparing these calculated values with the measured concentrations shows (Table 1) that only 0.8% (L261) to 7% (L224) of the added ^{226}Ra was in the lake water in 1981.

^{226}Ra profiles over 1 yr (Fig. 1) show that while surface concentrations are quite stable, varying only about 20%, deepwater concentrations in lakes that have partial anoxia show a pronounced seasonal cycle. Complete oxygen data are shown with the ^{226}Ra data for L227 and L226S. While oxygen data for L261 and L224 are not available for 1980–81, data from other years (D. Schindler, Freshwater Institute, Winnipeg, Man., unpubl. data) show significant anoxia in the hypolimnion of L261 and only occasional anoxia at the greatest depth in L224.

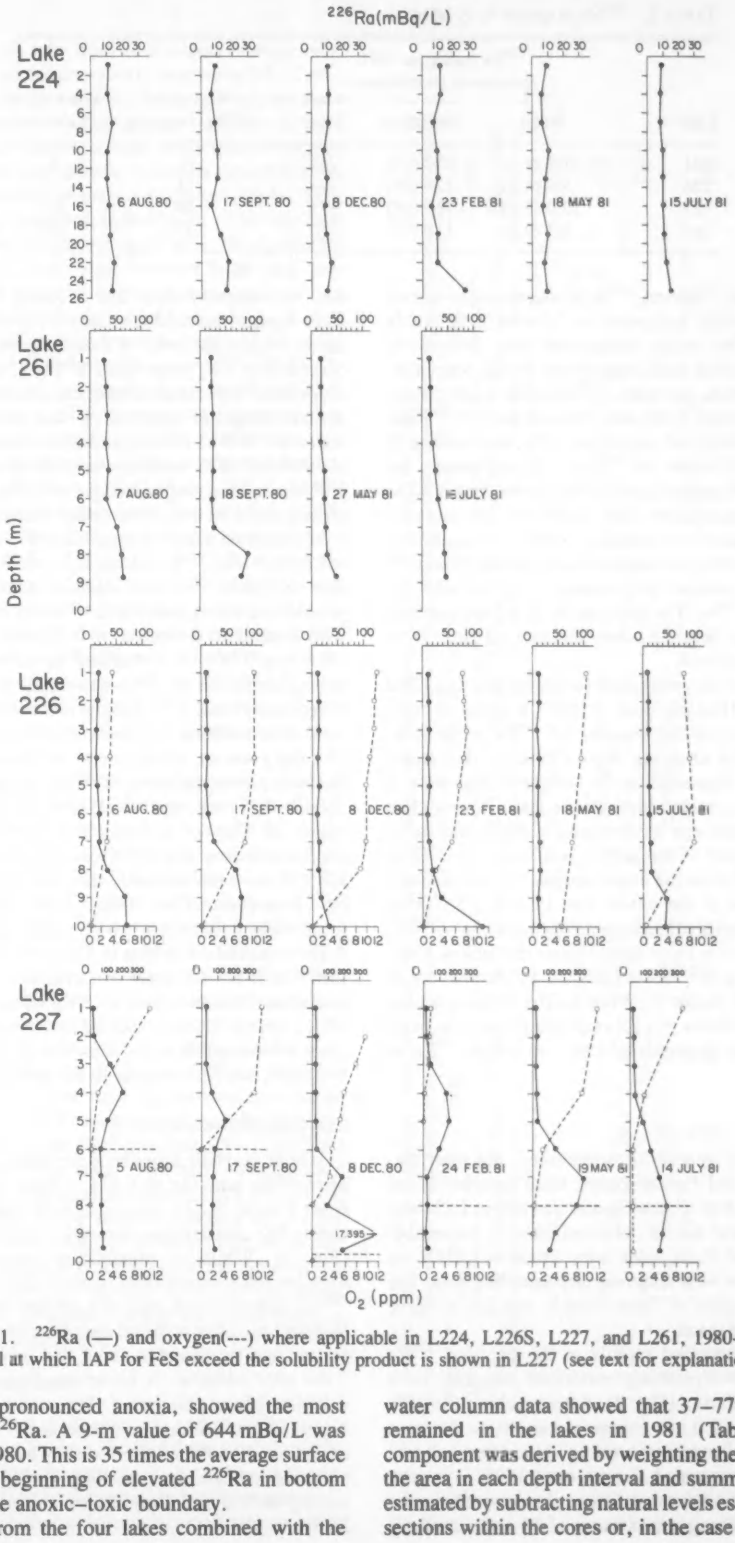

FIG. 1. ^{226}Ra (—) and oxygen(---) where applicable in L224, L226S, L227, and L261, 1980–81. Level at which IAP for FeS exceed the solubility product is shown in L227 (see text for explanation).

L227, with the most pronounced anoxia, showed the most pronounced effect on ^{226}Ra. A 9-m value of 644 mBq/L was reached in December 1980. This is 35 times the average surface value. In all cases the beginning of elevated ^{226}Ra in bottom water coincides with the anoxic–toxic boundary.

Analyses of cores from the four lakes combined with the water column data showed that 37–77% of the added ^{226}Ra remained in the lakes in 1981 (Table 2). The sediment component was derived by weighting the total excess radium by the area in each depth interval and summing. Excess ^{226}Ra was estimated by subtracting natural levels estimated from both deep sections within the cores or, in the case of L226S, cores taken

TABLE 2. ^{226}Ra in spiked ELA lakes.

Lake	^{226}Ra (mBq) in 1981 (percent in parentheses) Water	Sediments	Total	% of added ^{226}Ra remaining	Years since addition
224	27.0 (9.4)	259 (91)	286	77	5
226	5.6 (4.2)	126 (96)	131	71	3
227	10.0 (5.7)	167 (94)	176	49	11
261	3.3 (9.4)	118 (97)	122	37	10

prior to ^{226}Ra addition. Natural ^{226}Ra concentrations varied from 2.0 mBq/g in sandy sediments to 55 mBq/g in highly organic sediments. The water component was derived by summing volume-weighted strata represented by the concentrations of ^{226}Ra (8–10 strata per lake). ^{226}Ra in the water ranges from 2.7 to 9.5% of the total in the lake. The estimate of ^{226}Ra in the water has an uncertainty of only about 10%, but because of the heterogeneous distribution of ^{226}Ra in the sediments, the estimate of total ^{226}Ra is probably not better known than ±30%. These estimates of uncertainty are based on the variance encountered in duplicate or multiple cores. The sediment integral for L227 is probably an underestimate because the cores taken in shallow water were not long enough to capture all of the sediment with excess ^{226}Ra. The estimate for L261 sediments is subject to a larger error than the others because no cores from shallow water were analyzed.

Because of the intense sampling done for previously described experiments in L224 (Hesslein et al. 1980b), a series of cores was available to determine the increase of ^{226}Ra in the sediments with time. These analyses show (Table 3) that added ^{226}Ra is preferentially deposited on the sediments that were in contact with the surface mixed layer of the lake. A very clear break in ^{226}Ra deposition can be seen over a depth interval of only 1 m in the early part of the series, with only 10–20% as much being deposited below the mixolimnion. This areal distribution persists in time to the extent that in 1981, 5 yr after ^{226}Ra addition, the mean areal sediment concentration of ^{226}Ra in less than 7 m of water is more than 3 times that below 15 m. This present partitioning of ^{226}Ra in L224 can be compared with that in L226S and L227 (Table 4). While L226S shows a similar pattern to L224, L227 shows just the opposite proportion, with deep sediments having accumulated twice as much ^{226}Ra as shallow sediments.

Macrophytes

The three dominant genera of submerged macrophytes, *Lobelia*, *Eriocaulon*, and *Potamogeton*, were sampled in the depth range of 0.25–1.5 m. *Potamogeton* was rare in L224 and a sufficient sample could not be collected after 30 September 1976. Only *Lobelia* and *Eriocaulon* were found in L226S. In most cases, five samples were taken on each sampling date. The average standard deviation of these sample sets for all three species was 21% of the mean.

All three species responded rapidly to the elevated ^{226}Ra concentrations in L224, reaching maximum concentrations within 20 d of ^{226}Ra addition (Fig. 2). After reaching the peak value the concentrations fell in a manner similar to the decrease of dissolved ^{226}Ra until reaching a stable value after 3–4 mo. This level was then maintained at least until 1978, 2 yr after ^{226}Ra addition. While *Lobelia* and *Eriocaulon* showed similar values throughout, reaching a maximum of 44 and 52 mBq/g wet wt, respectively, and leveling out at 11–15 mBq/g, *Potamogeton* reached a value of 265 mBq/g and remained at about 4 times the level of the other two genera until sampling ceased. The long-term value of 11–15 mBq/g for *Lobelia* and *Eriocaulon* represents a bioaccumulation factor (ratio of ^{226}Ra per wet weight of organisms to that in an equal weight of lake water) of 1100–1450 (assuming the concentrations in the water in 1978 and 1977 were similar to those reported here for 1980). If *Potamogeton* maintained a concentration 4 times that of the other genera, its bioaccumulation would have been about 5000.

Macrophyte samples from L226S were available from both before and after ^{226}Ra addition; however, a more limited record was available after the addition than in L224. The mean preadditive concentration in *Lobelia* was 1.2 mBq/g wet wt with a standard deviation of 0.30 mBq/g. For *Eriocaulon* the value was 0.96 with a standard deviation of 0.30. As in L224, the accumulation of ^{226}Ra was rapid. The concentration (Table 5) reached values >37 mBq/g 1–2 mo after ^{226}Ra addition. By 3 mo after addition the concentrations had dropped to 23 and 32 mBq/g wet wt, respectively, for *Lobelia* and *Eriocaulon*. By this time, concentrations of ^{226}Ra in the water had dropped to 55 mBq/L (Torgersen et al. 1982), giving an accumulation factor of 420 for *Lobelia* and 570 for *Eriocaulon*. If the concentrations in macrophytes continued at this level, as seen in L224 from 3 mo onward, the bioaccumulation factor would have been about 2000–3000 in 1981. Probably the true value is somewhere in between, which makes the L226S bioaccumulation comparable with that in L224. While no preaddition value for ^{226}Ra in L226S water was available, a good estimate can be made based on other natural ^{226}Ra values in ELA lakes. A value of 1.5 mBq/L (from a lake of similar morphometry to L226S) gives a bioamplification factor for preaddition macrophytes of 650–800, which is similar to the postaddition values.

Crayfish (*Orconectes virilis*)

Whole crayfish samples were taken over a period of 3 mo after ^{226}Ra addition to L224. Three animals, ranging in size from 1.48 to 5.62 g fresh wt, were caught on most sampling dates. The crayfish also showed a rapid accumulation of ^{226}Ra (Fig. 3). While no preaddition samples were taken, early samples and samples from control L239 suggest that the natural ^{226}Ra concentration was not greater than 20 mBq/g wet wt. Within 1 mo after addition the concentration rose to 370 mBq/g wet wt and remained at about that level until sampling ended 3 mo after addition. A bioaccumulation factor of 750 can be calculated from this value. Variance among sample sets was higher in crayfish than macrophytes, with an average standard deviation of 54% of the mean. This may be caused by the variation in age, sex, molting cycle, and/or feeding habits among individuals. Crayfish are not found in L226S, L227, or L261.

Fish

^{226}Ra analyses done on lake trout (*Salvelinus namaycush*), white sucker (*Catostomus commersoni*), lake whitefish (*Coregonus clupeaformis*), pearl dace (*Semotilus margarita*), northern redbelly dace (*Chrosomus eos*), and fathead minnow (*Pimephales promelas*) (Table 6) showed that in all cases, samples taken after ^{226}Ra addition had greatly increased concentrations when compared with preaddition and control lake samples. Fish heavier than 50 g were analyzed individually. The standard deviations within sample groups averaged 36%. No significant differences in variance were seen between high and low concentration groups. Dace and minnows were analyzed in batches to achieve a total sample weight of >50 g. Within sample groups, which usually had a weight range of not more than a factor of 2–3, no correlation between ^{226}Ra and body weight was found. However, two small lake trout (68 and 70 g) caught in L224 in the fall of 1978 showed consistently higher ^{226}Ra concentrations than larger fish caught on that date. No consistent difference was found in ^{226}Ra concentrations between large lake trout and white suckers from L224 in the fall of 1980. For all catches of lake trout and white sucker in L224, except for the two small fish mentioned, the bioaccumulation factor range was only 44–57. For the two small lake trout the factor was 154. These postaddition values for ^{226}Ra in L224 can be contrasted with values from control (no radium addition) L223 and L239. Lake trout in L223 had concentrations of less than the detection limit of 20 mBq/kg wet wt for 300 g of fish. White suckers had an average concentration of 108 mBq/kg. This gives a bioaccumulation factor of 78.9. Lake trout in L239 had an average ^{226}Ra concentration of 25 mBq/kg and a bioamplification factor of 45.2.

Lake whitefish showed a response similar to lake trout to ^{226}Ra in the lake water. Preaddition lake whitefish from L226S had ^{226}Ra concentrations of 37 mBq/kg wet wt, as did the single lake whitefish from L226N. Lake whitefish in unspiked L302S had concentrations averaging 81 mBq/kg (Elliott et al. 1981). These concentrations represent bioaccumulation factors of 27.5 in L226S and 45.7 in L302S. Three months after addition of ^{226}Ra, lake whitefish in L226S had 478 mBq/kg, representing a bioaccumulation of 47.8. It appears, then, that for lake trout, white sucker, and lake whitefish the bioaccumulation factor, which range from 30 to 80, has no clear dependence on ^{226}Ra concentration in water and is similar for all three species.

Pearl dace, northern redbelly dace, and fathead minnows taken from L227 and L224 show much higher bioaccumulation factors, ranging from 235 to 637 for northern redbelly dace and 743 for fathead minnows, while pearl dace had 320–1156 times the ^{226}Ra concentration in the water. Northern redbelly dace from control L302S had a bioaccumulation factor of 610 but pearl dace had only 184. These bioaccumulation factors are approximately 10 times those found in the larger lake whitefish, lake trout, and white suckers and are in the same range as the bioaccumulation factor for macrophytes. The L227 concentrations of 4–20 mBq/g wet wt are 20–50% of the values found in L227 sediments of the mixolimnion.

Discussion and Conclusions

The gross geochemical behavior of added ^{226}Ra in a Canadian Shield Lake can be described by three processes: the rapid removal of ^{226}Ra from the water to the surficial sediments, the slower decrease in ^{226}Ra concentration by water renewal, and long-term burial by sediment accumulation (Emerson 1974;

TABLE 3. Excess ^{226}Ra (mBq/cm^2, integrated with background subtracted) in L224 sediment cores at different water depths.

Water depth (m)	1976						1977					1978		1981, Mar.–May
	3 Aug.	13 Aug.	20 Aug.	26 Aug.	21 Sept.	14 Dec.	31 Mar.	16 June	16 July	1 Nov.	14 Dec.	13 Feb.	24 June	
0												122	99/83	131
1	22	50										91/144		124
2				55	104							83/119	94/97	
3	39	65					139/114	255/126			111/144	181	215/62	148
4			49	84	75						92			118
5	39	53					115/242	133/101			130		84/62	176
6			41	97	95		228/244							
7	4	11		37			192	107/77			158		148/145	
8			53	39	29						118			198
9													212/144	
10			10					48/51					149/104	
15						73				51			59/79	77
							19							
			4		2					41		68	10	46
						31			45					
										21				
25			3		6	8	32			44	24	25/26	69	28

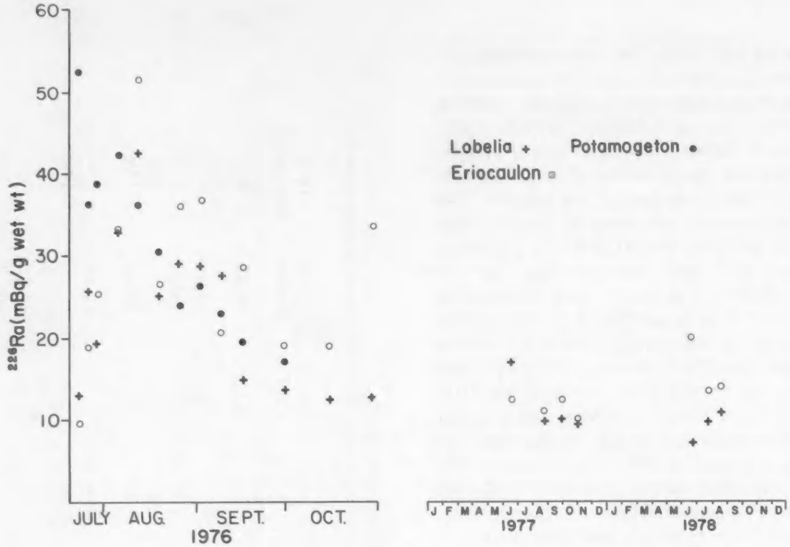

FIG. 2. ^{226}Ra in macrophytes from L224 for 1976, 1977, and 1978.

TABLE 4. Relative areal deposition of ^{226}Ra in sediments.

Lake	Core depths used shallow/deep (m)	Ratio of ^{226}Ra integral shallow/deep cores
224	1, 2, 3, 4, 5, 6, 7/16, 20, 27	3.2
226S	1, 2, 3/8, 11	5.3
227	1, 2, 3, 4, 5/16, 10	0.44[a]
261	No shallow cores analyzed	

[a] Amounts in shallow cores are probably underestimates (see text); the true ratio is probably closer to 0.6.

TABLE 5. ^{226}Ra (mBq/g) in macrophytes from L226S.

Date	*Lobelia*	*Eriocaulon*
Preaddition		
31 Oct. 1977	0.78	—
17 May 1978	1.07	1.04
15 June 1978	—	1.11/1.07/0.92
20 July 1978	1.52	0.70
^{226}Ra addition (20 July 1978)		
17 Aug. 1978	45.5	34.8
14 Sept. 1978	46.3	42.9
17 Oct. 1978	23.3	31.5

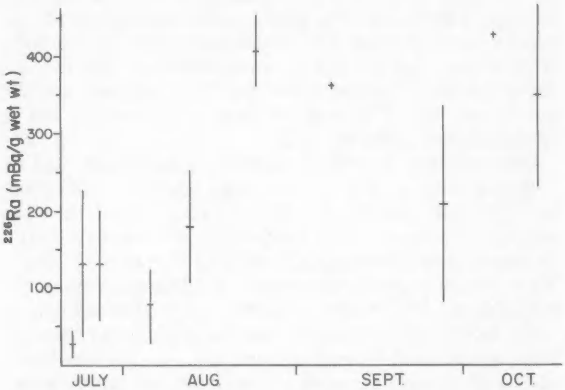

FIG. 3. ^{226}Ra in crayfish (*O. virilis*) from L224, 1976. Vertical dimension of cross represents standard deviation or range if only two samples were taken.

Hesslein et al. 1980a; Torgersen et al. 1982; Santschi et al. 1983). The rapid removal can be adequately represented by a first-order equation, as shown for several metal isotopes (Hesslein et al. 1980a), which in this case would be

$$(1)\quad {}^{226}\mathrm{Ra}_t = {}^{226}\mathrm{Ra}_{t=0}e^{-Kt}$$

where K is the removal coefficient. The value of this coefficient for the four lakes studied has been reported to be in the range of 1–3.5% per day (half-times of 20–60 d) (Emerson 1974); Hesslein et al. 1980b; Torgersen et al. 1982). A small modification can be made to equation (1) to allow for some residual value to be reached rather than a continual decline. This can be represented by

$$(2)\quad {}^{226}\mathrm{Ra} = ({}^{226}\mathrm{Ra}_{t=0} - {}^{226}\mathrm{Ra}_{t=\infty})e^{-Kt} + {}^{226}\mathrm{Ra}_{t=\infty}.$$

The residual value appears to be determined by some exchange with the sediments as it persists through several lake water residence times. The residual level in the water is 3–9% of the total ^{226}Ra in the lakes (Table 2).

The mean long-term loss rate of ^{226}Ra in the water column and that exchangeable from sediments by water renewal can be calculated using the present fraction of added ^{226}Ra (Table 2) remaining in the lakes. If one assumes the system to act as a well-mixed box with outflow concentrations representative of lake concentrations and that exchange with the sediments is rapid as represented by K from equation (1), a first-order

TABLE 6. Summary of ^{226}Ra in fish in ELA lakes.

Lake	N	Type	Date	Weight range (g)	^{226}Ra (Bq/kg wet wt) ± SD	Bioaccumulation factor[c]
224	2	Lake trout	Fall 1978	68, 70	1.28, 135	154
	3	Lake trout	Fall 1978	220–530	0.48±0.19	56.9
	13	Lake trout	Fall 1980	475–795	0.38±0.16	44.3
	6	Lake trout	Fall 1980	435–715	0.44±0.20	51.3
	5	White sucker	Fall 1980	1100–1665	0.47±0.12	55.2
	36	Pearl dace	Fall 1982	1.9–3.1 (Σ = 89.6)	3.08	362
	16	Pearl dace	Fall 1982	7.2–13.3 (Σ = 161.5)	2.73	320
	11	Fathead minnow	Fall 1982	1.9–3.0	6.33	743
226S	2	Lake whitefish	Fall 1977	530, 600	0.044[a]	29.5
	4	Lake whitefish	Spring 1978	510–765	0.040±0.011[a]	26.8
	6	Lake whitefish	Fall 1977 Spring 1978	510–765	0.041±0.013[a]	27.5
	4	Lake whitefish	Fall 1978	605–765	0.48±0.17	47.8
226N	1	Lake whitefish	Fall 1978	607	0.043[a]	29.3
223	4	Lake trout	Fall 1978	26–279	<0.019[a]	<13.5
	7	White sucker	Spring 1981	240–800	0.108±0.029[a]	78.9
239	4	Lake trout	Spring 1981	2227–4550	0.025±0.008[a]	45.2
227	67	Northern redbelly dace	Summer 1981	Σ = 107.1	7.25	426
	43	Northern redbelly dace	Summer 1981	Σ = 70.6	10.8	637
	40	Northern redbelly dace	Summer 1981	Σ = 68.6	4.00	235
	21	Pearl dace	Summer 1981	Σ = 48.6	19.7	1156
302	10	Lake whitefish	Summer 1982	120–360	0.081±0.056[b]	45.7
	18	Pearl dace	Summer 1982	Σ = 102.2	0.27	184
	36	Northern redbelly dace	Summer 1982	Σ = 52.2	0.90	610

[a] ^{226}Ra not added to these lakes or samples prior to addition.
[b] Taken from Elliott et al. (1981).
[c] See text for explanation.

equation again applies giving

$$(3)\quad \Sigma\,^{226}\text{Ra} = \Sigma\,^{226}\text{Ra}_{t=0}e^{-\alpha t}$$

where α is the coefficient of loss from the total lake (sediment and water). This can be solved as

$$(4)\quad -t^{-1}\ln\frac{\Sigma\,\text{Ra}}{\Sigma\,\text{Ra}_{t=0}} = \alpha.$$

Substituting values from Table 2 gives loss rates of 0.052/yr for L224, 0.11/yr for L226S, 0.065/yr for L227, and 0.099/yr for L261.

A direct approximation of the 1981–82 loss rate due to water renewal can be made based on the fraction of ^{226}Ra presently in the water (residual value of equation (2) and the actual water renewal times of the lakes. The water renewal coefficient is the product of reciprocal of the water residence time, T_r^{-1}, and the fraction in the water, f. The mean long-term loss coefficients α from equation (4) exceed the calculated renewal model coefficients by factors of 3–13 (Table 7). This discrepancy is probably due to the fact that f was initially unity and decreased to its present value over the 1st yr or so after addition as described by equation (2). This would have caused a larger loss early in the experiment. The decrease in α compared with fT_r^{-1} with the age of the ^{226}Ra addition (Table 7), probably due to decreased importance of initial conditions, supports this explanation. Long-term loss of ^{226}Ra in the four lakes can be expected to be on the order of 0.5–2% per year in the future as predicted by the water renewal model.

TABLE 7. Long-term loss of ^{226}Ra by water renewal (see text for explanation)

Lake	t (yr)	T_r (yr)	f	fT_r^{-1} (yr^{-1})	α (actual)	α/fT_r^{-1}
224	5	15.3	0.094	0.0061	0.052	8.5
226S	3	5	0.042	0.0084	0.11	13.1
227	11	2.6	0.057	0.022	0.065	3.0
261	10	1.3	0.027	0.022	0.099	4.5

The concept of a distribution coefficient between water and sediments (K_d) (Duursma and Hoede 1967, 1970) is not useful in predicting the equilibrium as steady-state concentration of ^{226}Ra in the water (Benes 1982). K_d based on weight fails because of the high affinity of radium for the organic part of the sediments (56 mBq/g in highly organic sediments vs. 2.0 mBq/g in sandy sediments in L224). Because the areal deposition of ^{226}Ra in the sediments varies much less than the concentration per weight, the amount of water per unit area of sediment relates better to the proportion of ^{226}Ra in the water column. There is very good correlation ($r = 0.953$, $P < 0.01$) between the mean depth and the ratio of ^{226}Ra in water to ^{226}Ra in sediment (Fig. 4). The importance of this relationship is that the residence time of radium in deep lakes should not be as long as might be expected from their generally longer water residence times. Studies on a broader range of lake sizes are warranted because this relationship could be very important in producing a general model for radium flushing from lake sediments.

Because radium does not change oxidation state in anoxic

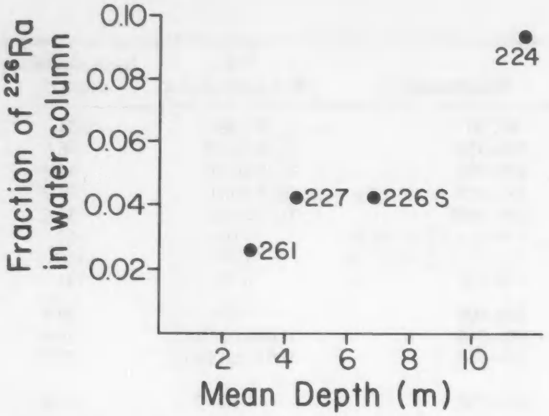

FIG. 4. Fraction of the total ^{226}Ra in each lake that was in the water column vs. the mean depth of the lake.

waters, the increased concentrations observed in L227, L226, and L261 must be explained by desorption rather than increased solubility. Manganese oxides are the most likely candidates for radium sorption. Their very high affinity for radium is well documented (Moore and Reid 1973). Manganese is relatively easily reduced in natural anoxic waters and is often found in the reduced form (Mn^{2+}) even in the presence of a small amount of oxygen. These characteristics would explain the rapid rise in ^{226}Ra concentration slightly above the level of zero oxygen. When the lake water is well oxygenated in the fall, ^{226}Ra values return to lower levels, probably by readsorption to newly precipitated manganese oxides.

The radium cycle in L227 (Fig. 1) is further characterized by a decrease in concentration in the deepest levels of the lake. This decrease follows the previously described increase resulting in a mid-depth maximum. Dissolved manganese and iron in L227 (D. Schindler, unpubl. data) show a continuous increase in concentration to the maximum depth throughout the anoxic periods. However, there is clear evidence for precipitation of iron sulfides (Cook 1981) in L227. Ion activity products (IAP) for FeS were calculated based on Cook's method using iron data of Schindler and sulfide calculated as the "missing sulfate" (Cook 1981) relative to fall or spring mixing values. The shallowest depth at which FeS IAP is exceeded is shown by the broken line in Fig. 1 (L227). Because the data were available at 2-m intervals only, the actual level could be as much as 2 m shallower. The lines generally coincide well with the upper level of the radium depletion. This suggests a possible coprecipitation or adsorption of radium to precipitated FeS. There is no evidence for FeS precipitation in either L226S or L261, which show no radium depletion in the anoxic waters.

Although the concept of bioaccumulation has often been used in aquatic food chain studies (Anderson et al. 1963; Williams 1982; Rope 1981), its utility with radium is limited. Extensive knowledge of food chains is necessary for proper interpretation of data, and little information on physiological processes can be extracted. In the case of radium accumulation, a more effective approach is to compare radium and calcium concentrations. Although this has been done infrequently for radium in organisms (Rope 1981; Marple and Potter 1982; Vanderploeg et al. 1975), much literature is available on the relative uptake and concentration of strontium (also a group IIa element) and calcium. The fundamental principle in this approach is that radium (or strontium) follows the same biologic pathways as calcium but is favored or discriminated against to a greater or lesser extent in different processes or organisms. The selection for or against radium as compared with calcium is expected to be greater than for strontium because of the larger differences in atomic weight, radii, etc.

Berg (1968) and Ophel and Judd (1967) described two pathways controlling strontium and calcium accumulation and exchange in freshwater fish: (1) direct absorption, probably through the gills, and (2) ingestion in food. Ingestion from water is negligible because freshwater teleosts do not drink. Both of the above studies demonstrate that accumulation of labeled strontium from both water and food is directly proportional to the concentration of strontium in the water and food. Berg (1968) further demonstrated that calcium exchange is proportional to calcium concentration in water and food. Since strontium is taken up with calcium, the body burden of strontium in the fish is dependent on the strontium/calcium ratio in the water and food as well as the relative calcium uptake due to ingestion versus gill exchange. Discrimination against strontium is greater in gill exchange than in ingestion (Berg 1968; Ophel and Judd 1967). The discrimination factor, called OR (for "observed ratio") is expressed as

$$\left(\frac{\text{Sr or Ra in sample}}{\text{Ca in sample}}\right) \Big/ \left(\frac{\text{Sr or Ra in water}}{\text{Ca in water}}\right).$$

The discrimination in gill exchange has been determined to be 0.2–0.4 and 0.7–1.0 from food (Ophel and Judd 1976; Berg 1968; Ichikawa 1960). Ophel and Judd (1976) showed that fish gained from 33% of their strontium from water when they obtained most of the calcium from food to 91% of their strontium from water when they obtained little calcium from food. Berg (1968) presented data from a number of authors in which fish in natural waters showed OR of 0.2–0.4, suggesting that the primary pathway was exchange from water.

The OR's (Table 8) demonstrate the considerable discrimination against ^{226}Ra in fish relative to their surroundings. With the exception of the two dace species and fathead minnows, the OR's fall between 0.011 and 0.032. This range represents an order of magnitude greater discrimination against radium as compared with strontium. A possible explanation for the much higher values for dace and fathead minnows may be their dietary intake of radium. These dace and fathead minnow species eat periphyton and detrital material. In L227 this material has a radium/calcium ratio of 33 000, about 5 times that in the water. In addition, if radium follows the strontium/calcium pattern, discrimination in dietary intake would be lower. Because these fish are small and fast growing they may show a greater influence of dietary radium than the other larger, slower growing fish.

In another study of radium and calcium in trout, Rope (1981) found OR's ranging from 0.058 to 0.93 in four different uranium mine settling ponds. This represents a somewhat lower discrimination against radium than found in this study. Some of the factors that could explain the differences are the different species of fish (rainbow trout, *Salmo gairdneri*, and brook trout, *Salvelinus fontinalis*), the age of the fish, and the unnatural sediment and water chemistry of the pond; Rope's fish were 0.5–4 yr of age, while most large fish in this study were >5 yr old and possibly some as old as 30 yr (K. Mills, Freshwater

TABLE 8. OR (observed ratio) Ra vs. Ca in organisms and lake water.

Sample	Ca (μmol/g wet wt)	Ra/Ca (Bq/mol) Organisms	Ra/Ca (Bq/mol) Lake water	OR
L224 lake trout	80	5.33	189	0.028
L224 pearl dace	208	13.9	189	0.079
L224 fathead minnow	200	31.6	189	0.167
L224 white sucker	170	2.78	189	0.015
L226S lake whitefish	93	5.14	222	0.023
L227 pearl dace	208	94.5	252	0.376
L227 northern redbelly dace	224	42.4	252	0.168
L223 lake trout	80	<0.22	28.3	<0.008
L223 white sucker	170	0.64	28.3	0.023
L226N lake whitefish	93	0.44	30.0	0.015
L302 lake whitefish	93	0.87	37.0	0.024
L302 pearl dace	208	1.31	37.0	0.035
L302 northern redbelly dace	224	4.03	37.0	0.019
L239 lake trout	80	0.31	6.25	0.050
L224 *Eriocaulon*	8.2	1800	189	9.55
L224 *Lobelia*	11.1	1000	189	5.29
L224 *Potamogeton*	24.1	2760	189	14.6
L226 *Eriocaulon*	8.2	113	30.0	3.76
L226 *Lobelia*	11.1	100	30.0	3.33
L224 Crayfish	655	522	189	2.76
L224 Crayfish	655	<18.5	11.3 (estimated)	<1.67

Institute, Winnipeg, Man., pers. comm.). Rope reported very little difference between OR determined in bone, skin, and muscle but found a strong positive correlation with age of fish; however, this correlation was contrary to expectations and may have been a spurious result.

Some values of bioaccumulation factors for radium in fish have been reported but cannot be properly compared because no calcium values were given. Anderson et al. (1963) presented values of 3–4 for fish flesh and ~100 for fish bone from the Animas River in Colorado. Schüttelkopf and Kiefer (1982) listed a value of 18 for fish from the Black Forest of Germany.

Comparison of OR's for radium in macrophytes (Table 8) with those for strontium again shows a greater discrimination factor, this time, however, in favor of radium. Ophel and Fraser (1970) presented OR's for strontium in a number of vascular aquatic plants from Perch Lake, Ont. Of 22 species they found 16 that discriminate against calcium (OR > 1) and five with OR > 2. Radium postaddition values in L224 range from 5.3 to 14.6, whie preaddition estimates for L226 are 3.3 and 3.8. Ophel and Fraser (1970) analyzed two algal species and showed similar OR's for strontium. Rope (1981) reported values for radium OR of 10–60 for algae and 1.7–18 for vascular plants in uranium mine settling ponds. These OR's cover nearly the same range of values presented here. Marple and Potter (1982) found radium OR's of 0.5–2.6 for terrestrial plants in uncontaminated (63–260 mBq/g) and uranium mill site (6–30 Bq/g) soils. These results suggest that fast-growing herbivorous fish should show the highest radium/calcium ratios.

There are no data in the literature for strontium/calcium in crayfish; however, a value of 10 can be calculated from the data of Schurr and Stamper (1962) assuming a weight per animal of ~3 g. The OR's (Table 8) for radium are 2.8 in L224 after radium addition and are estimated to be <1.8 at the natural levels. The value of 2.8 is probably an overestimate because the samples were taken only ~100 d after ^{226}Ra addition, before a steady-state value was achieved. Calcium uptake, which is very rapid during molting, is primarily from the water (McWhinnie 1962; Greenaway 1974; Malley 1980).

In conclusion, we will summarize our present understanding of the geochemical and biological movement of radium in Canadian Shield lakes. Ionic dissolved radium added to the lake is not sorbed to particles to a great extent (<10%) but does sorb to the sediments with a time constant of 0.01–0.05/d. In a stratified lake, sorption is limited to sediments in contact with the altered waters. Long-term sediment redistribution and sorption–desorption result in a more uniform distribution of radium in sediments. Radium is sorbed to sediments to the extent that only 3–10% remains in the water column. This fraction is positively correlated with mean depth. As a result of the small fraction remaining in the water, losses from the whole lake (sediment and water) are less than 10% per year.

Biological components in the lakes respond rapidly to the increased radium concentration (days to weeks). The biological uptake is strongly linked to calcium metabolism. Radium is favored over calcium in macrophytes and crayfish but is discriminated against strongly in fish. Concentrations of radium in fish are controlled primarily by the concentration in the water, but small, fast-growing fish or those with a very high radium food source can be influenced by radium in the food. The implication of the above scenario is that due to the characteristically low calcium concentrations in these lakes and relatively long water residence times, biota in Canadian Shield lakes have a high potential for radium uptake from mining of uranium deposits that are common in the formation.

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Changes in the Selection of Microhabitat by Juvenile Atlantic Salmon (*Salmo salar*) at the Summer–Autumn Transition in a Small River

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Over three summers we used direct underwater observation to examine the summer to autumn differences in seven microhabitat properties of three age-classes of juvenile Atlantic salmon (*Salmo salar*) in the Little Sevogle River of northeastern New Brunswick. Salmon of all three age-classes occupied a wide range of water depths during summer, but were concentrated mainly in depths of 24–36 cm. In autumn, they occurred in this range almost exclusively. The streambed stones most closely associated with the individual positions of all ages were always <20 cm in summer and mostly (84–92%) <10 cm in diameter. In autumn, all ages were associated with home stones up to 40 cm in diameter, with 65–83% of the stones exceeding 20 cm; the size of home stones selected increased with fish age in autumn. There was no apparent relationship between the water depth and home stone size distributions occupied by all age-classes and available in the stream during either summer or autumn. Summer focal water velocity (velocity at the fish's snout) was predominantly 10–30 $cm \cdot s^{-1}$ for 0^+, 10–40 $cm \cdot s^{-1}$ for 1^+, and 30–50 $cm \cdot s^{-1}$ for 2^+ salmon, but during autumn it was almost always <10 $cm \cdot s^{-1}$ for all ages. The bottom and surface water velocities as well as the maximum water velocity within 1 m of fish stations increased with fish age during summer and autumn. At the summer–autumn transition, 0^+ salmon selected higher bottom, surface, and maximum water velocities, 2^+ salmon selected lower velocities, but selection by 1^+ salmon remained unchanged. We view substrate size followed by water depth as the primary properties influencing stream suitability for juvenile Atlantic salmon in autumn.

Des observations directes sous l'eau ont servi durant trois étés à examiner les différences, entre l'été et l'automne, de propriétés de sept microhabitats de trois classes d'âge de saumons atlantiques (*Salmo salar*) juvéniles dans la Petite rivière Sevogle du nord-est du Nouveau-Brunswick. Les saumons des trois classes d'âge occupent une gamme étendue de profondeurs d'eau en été, mais se concentrent principalement dans des profondeurs de 24–36 cm. À l'automne, on les trouve presque exclusivement à ces profondeurs. Les pierres du lit le plus étroitement associées avec la position individuelle des saumoneaux de tous âges sont toujours <20 cm en été et en grande partie (84–92%) <10 cm de diamètre. À l'automne, tous les âges sont associés avec des pierres de diamètre allant jusqu'à 40 cm de diamètre, 65–83% dépassant 20 cm ; la grosseur des pierres choisies pour leur territoire augmente en fonction de l'âge des poissons à l'automne. Il n'y a aucune relation apparente entre la profondeur de l'eau et la distribution de la grosseur des pierres du territoire occupé par toutes les classes d'âge et accessibles aux saumons dans le cours d'eau en été ou à l'automne. La vitesse focale du courant en été (vitesse au museau du poisson) est en grande partie de 10–30 $cm \cdot s^{-1}$ pour les poissons d'âge 0^+, de 10–40 $cm \cdot s^{-1}$ pour ceux de 1^+ et de 30–50 $cm \cdot s^{-1}$ pour les poissons d'âge 2^+, mais à l'automne, la vitesse est presque toujours <10 $cm \cdot s^{-1}$ pour tous les âges. La vitesse du courant au fond et à la surface, aussi bien que la vitesse maximale à moins de 1 m des positions occupées par les poissons, augmente en fonction de l'âge de ces derniers en été et à l'automne. Au moment de la transition entre été et automne, les saumons d'âge 0^+ choisissent des vitesses de courant de fond, de surface et maximale plus élevées, ceux d'âge 2^+ des vitesses inférieures, tandis que la sélection par les saumons d'âge 1^+ demeure la même. Selon nous, la taille du substrat et ensuite la profondeur de l'eau sont les principaux facteurs qui font qu'un cours d'eau convient aux jeunes saumons atlantiques en automne.

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Management of a fish population requires detailed knowledge of species habitat. Everest and Chapman (1972) showed that a number of individual microhabitat properties such as water depth, water velocity, and substrate size are very important to Pacific salmonids during the summer, and Bustard and Narver (1975) showed how Pacific salmon microhabitat selection changes as winter approaches. Furthermore, Mason (1976) found that a large increase in coho salmon (*Oncorhynchus kisutch*) biomass and potential smolt yield brought about by supplemental summer feeding was nullified by restricted winter carrying capacity. Riddell and Leggett (1981) suggested that severe winter conditions could result in genetic selection for autumn migration of large Atlantic salmon (*Salmo salar*) parr. Clearly, habitat management programs limited to summer observations are likely to be incomplete without detailed understanding of seasonal microhabitat requirements.

Rimmer et al. (1983) found that juvenile Atlantic salmon responded to the onset of winter conditions by sheltering beneath the substrate. The sudden movement into shelter was related to water temperature and occurred in areas of summer residence with little movement to other parts of the river. However, quantitative details of the microhabitat properties important to juvenile Atlantic salmon in any season are not conclusive in the literature. For example, during summer, 0^+ and 1^+ salmon may occupy water depths of 15–50 cm (Keenleyside 1962; Elson 1975; Jones 1975; Symons and Heland 1978; Egglishaw and Shackley 1982). Very wide ranges have also been reported for summer selections of substrate size (Elson 1975), water velocity (Symons and Heland 1978), and winter selection of water depths (Smirnov et al. 1976; Allen 1940) and water velocity (Smirnov et al. 1976). Clearly, more extensive and definitive information is needed on seasonal microhabitat selection by this species.

In this field study we investigated the seasonal changes in the microhabitat selection by juvenile Atlantic salmon. By examining and comparing microhabitat properties associated with individuals of three age-classes in a small northeastern New Brunswick river during summer and during autumn, we aimed to provide detailed information to extend present understanding of the seasonal behavior of this species.

Materials and Methods

Observations were made in a 5.6-km portion of the Little Sevogle River, a third-order tributary of the Miramichi River in northeastern New Brunswick. The Little Sevogle River rises at an altitude of 800 m and flows 39.5 km through mixed coniferous–deciduous forest, entering the Northwest Miramichi at sea level. Two study sites, A and B, were selected that were similar to one another and generally representative of the entire river. We used representative study sites to obtain results reflecting average microhabitat conditions in the river, thus avoiding possible influences introduced by studying individual extreme habitat areas (deep, shallow, slow, fast, etc.). Rimmer et al. (1983) provided general site descriptions.

Seasonal fluctuations in water temperature are great in the Little Sevogle River. During the summer (June–August), midafternoon temperatures range between 15 and 26°C. Temperatures decrease after August, reaching 0.1°C in mid-November when ice begins to form at the water surface. The water temperature remains near freezing (0.1°C) until mid-April. Frazil and anchor ice were not seen at either site.

Atlantic salmon did not spawn in either of the study sites although spawning areas existed directly upstream of both sites (50 m above site A; 325 m above site B).

To avoid the inevitable bias and disruption caused by electrofishing and netting, we observed fish directly using a wet suit, face mask, and snorkel. In summer, observation periods of 3-h duration were possible; but in late autumn cold water temperatures limited observations to 1–1.25 h. Microhabitat properties associated with an individual salmon were measured in both study sites for all fish observed within 0.5 m of transects crossing the river perpendicular to the water current at 6-m intervals. There were 27 transects at site A (total transect length = 192 m) and 21 at site B (total length = 252 m). Observations were made between 13:30 and 16:30 at approximately weekly intervals as weather and stream conditions permitted (stream well illuminated, sky 50% clear, underwater visibility ≥ 4 m). In 1976, observations were made during August 12–October 19, in 1977 during August 17–September 27, and in 1978 during May 31–November 24. The same transects were used during each observation.

During observation periods, each transect was followed in turn beginning at the downstream end of the study area. When a salmon was encountered it was watched for 3–5 min to determine the focal point of its territory (the location at which the fish spent the most time). Each fish was usually stationed over a single stone during the summer and beneath a single stone in the autumn (see Rimmer et al. 1983 for details). This was called the home stone. After the focal point had been found, fish length was determined and the following properties were assessed: water depth, home stone size, focal water velocity, bottom water velocity, surface water velocity, and maximum water velocity within 1 m of the focal point. These properties were chosen because of their importance to the shelter and food requirements of the fish. Measurements were made only if a fish remained undisturbed by the diver during the initial 3–5 min of observation. Then with care, the diver approached to measure the microhabitat properties at the focal point. Each fish could be approached to within 30 cm before it displayed any apparent distress. Although the fish retreated, they moved away from the focal point slowly, without indications of fleeing behavior. The focal point was reoccupied immediately after the diver had completed the measurements and moved away.

Fish length was determined by visually fixing against the substrate the positions of the anterior tip of the snout and the caudal notch. After gentle displacement of the fish, two scratches were made on the substrate to mark the length of the fish and the distance between them was measured. Comparison of mean lengths so measured with direct measurements of captured fish revealed a maximum error of 2%. Fish ages were then assigned according to length. During June–September, the three age-classes grew through the following length ranges: 0^+, 1.7–6.0 cm; 1^+, 5.0–10.0 cm; 2^+, 7.5–13.0 cm. Water depth was measured as the vertical distance from the water surface to the top of the home stone. Home stone size was determined by measuring the longest exposed dimension of the stone directly beneath the salmon during the summer and of the stone directly above the fish in the autumn.

Water flow velocities were measured with midget Bentzel velocity tubes (Everest 1967), with the modification that a flowmeter (Gilmont Instruments, Inc.) replaced the anemometer tube of the original design. They were calibrated against a pygmy Gurley current meter (Teledyne) and velocities of $9–80\ \mathrm{cm \cdot s^{-1}}$ could be measured with the three tubes used.

Where velocity was below the sensitivity of the tubes, it was recorded as being $<10\,\mathrm{cm \cdot s^{-1}}$.

Focal water velocity was measured at the focal point. It is the water velocity that the fish faced directly on its snout. Bottom water velocity was measured 5 cm above the home stone. Surface water velocity was measured 5 cm below the water surface directly above the focal point. Maximum water velocity was the greatest water velocity encountered at any depth within a 1-m radius upstream of the focal point; usually, velocity was maximal near the water surface.

Occupancy of the individual microhabitat properties was expressed as frequency distributions (number of fish at magnitude of property) and comparisons between sites, years, ages, and seasons were made by contingency log-likelihood ratios (Zar 1974). The frequencies were converted to percentages for illustration. Unless stated otherwise, results were considered not significant at $P > 0.05$.

Underwater visibility was about 8 m in bright sunlight, but on cloudy days it was reduced to about 4 m. Visibility was poor during spates, making observations at these times impracticable. Over a total of 148 h of direct underwater observation, 619, 266, and 175 observations were made of 0^+, 1^+, and 2^+ salmon, respectively. All measurements and notes were made immediately on an underwater writing slate. The transition from summer to autumn was taken as the time when midafternoon water temperature fell below 10°C in September. At this time the salmon moved into substrate chambers (Rimmer et al. 1983).

Results

The data from 1976–78 were pooled for each age-class at each site because there were no significant interyear differences in any of the microhabitat distributions. The pooled data for each site were then divided into two groups, summer and autumn. The frequencies with which each age-class was distributed over magnitudes of each microhabitat property did not differ significantly between sites during either season. Hence, data from both sites were pooled for each property.

The frequency distributions of water depth did not differ significantly between the three age-classes during either summer or autumn, and the mean depths occupied did not differ markedly between ages or seasons (Fig. 1). Fish were distributed over a wide range of depths during the summer, occupying depths from 6 to 54 cm. Marked peaks existed in the depth distributions at 24–36 cm; 74, 68, and 66% of the 0^+, 1^+, and 2^+ age-classes, respectively, occurred at these depths. In autumn, the depth range occupied was narrowed substantially to 18–42 cm, but the marked peaks remained at 24–36 cm where 91, 100, and 100% of 0^+, 1^+, and 2^+ age-classes, respectively, were found. The narrowing of the depth range occupied accounted for a significant difference ($P < 0.01$) between the summer and autumn distributions of each age-class. In neither season did the water depths occupied by the salmon approximate the water depths available to them.

The frequency distributions of home stone size adopted by the three age-classes were not significantly different during the summer. The respective mean home stone sizes during summer were 6.6, 6.7, and 6.4 cm for the 0^+, 1^+, and 2^+ fish. Summer home stones were never larger than 20 cm, and marked peaks occurred in the size distributions at <10 cm, with 84, 86, and 92% of the 0^+, 1^+, and 2^+ fish, respectively, having home stones of this size (Fig. 2). The frequency distribution of home stone sizes adopted in the autumn differed significantly ($P < 0.01$) from that adopted during summer for all age-classes. Most stones were in the range of 20–30 cm, with 65, 70, and 83% of 0^+, 1^+, and 2^+ fish, respectively, occurring beneath home stones >20 cm. In contrast with the summer situation, significant age-related differences ($P = 0.03$) occurred in the home stone size distributions adopted by the age-classes and, hence, sizes of fish. The respective mean home stone sizes for the 0^+, 1^+, and 2^+ age-classes in autumn were 17.8, 20.9, and 24.4 cm. The size distributions of home stones selected by all age-classes during the summer resembled the size distribution of stones

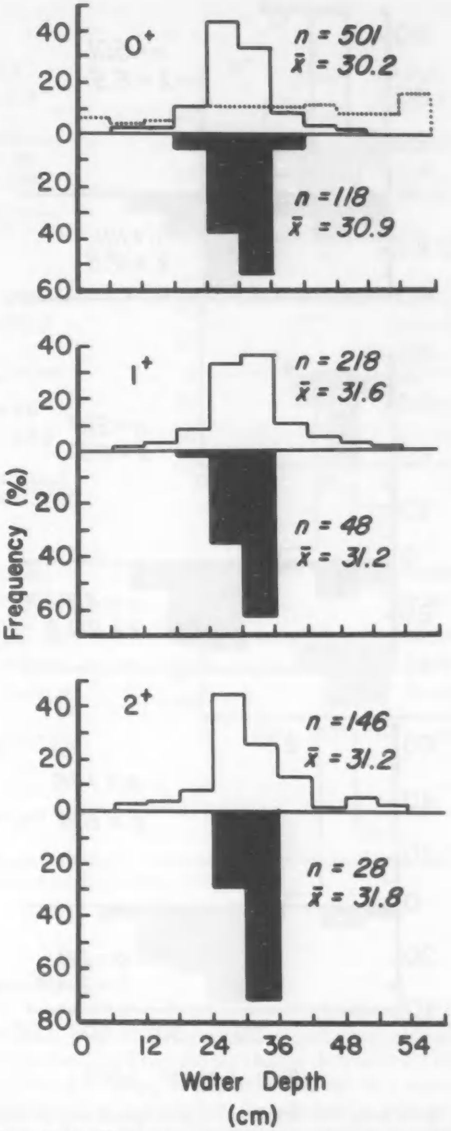

FIG. 1. Water depths occupied by three age-classes of juvenile Atlantic salmon in the Little Sevogle River during summer (white) and autumn (black), 1976–78. The dotted line in the 0^+ summer panel shows the distribution of depths available (1572 pooled measurements from both sites, and various gauge heights).

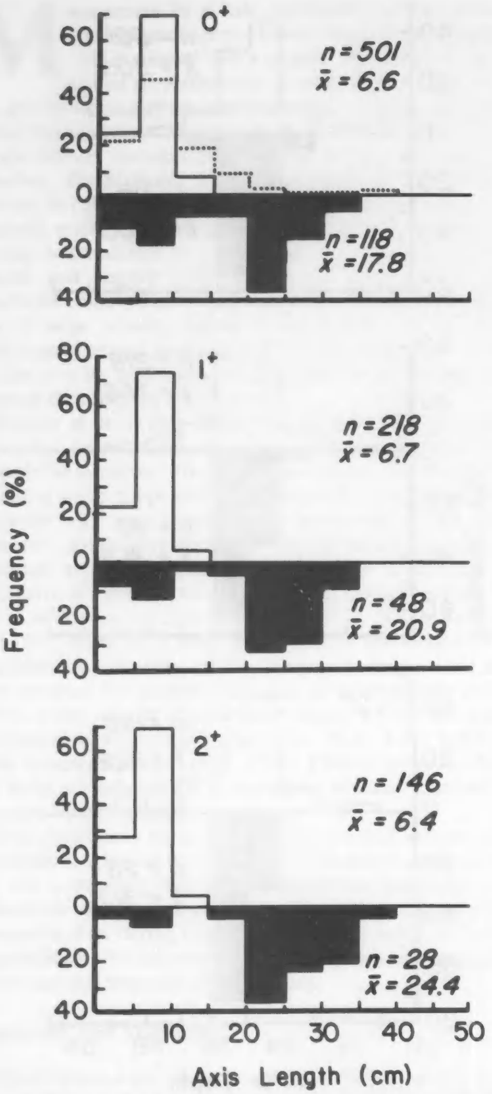

FIG. 2. Home stone size (length of longest exposed axis) associated with three age-classes of juvenile Atlantic salmon in the Little Sevogle River during summer (white) and autumn (black), 1976–78. The dotted line in the 0^+ summer panel shows the distribution of the surface substrate materials available (396 pooled measurements from both sites).

generally available on the streambed. In autumn, however, the distributions of selected and available home stones differed greatly, although the effect of availability persisted in the form of minor peaks at 5–10 cm.

In summer most salmon (96%) occupied illuminated areas, whereas in autumn, most salmon (74%) occupied shade. Only the few fish not sheltering in the substrate were recorded in illuminated areas in the autumn.

The distribution of focal water velocities occupied by salmon differed significantly ($P < 0.01$) between ages and, hence, size during the summer when 0^+ fish showed marked selection of

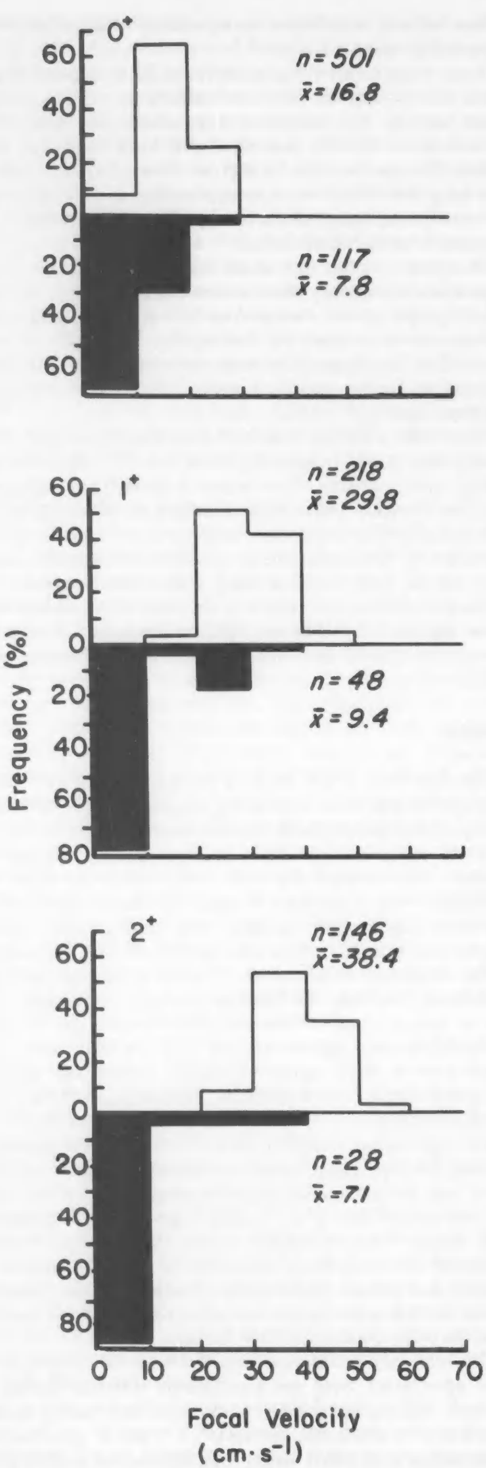

FIG. 3. Focal water velocity occupied by three age-classes of juvenile Atlantic salmon in the Little Sevogle River during summer (white) and autumn (black), 1976–78.

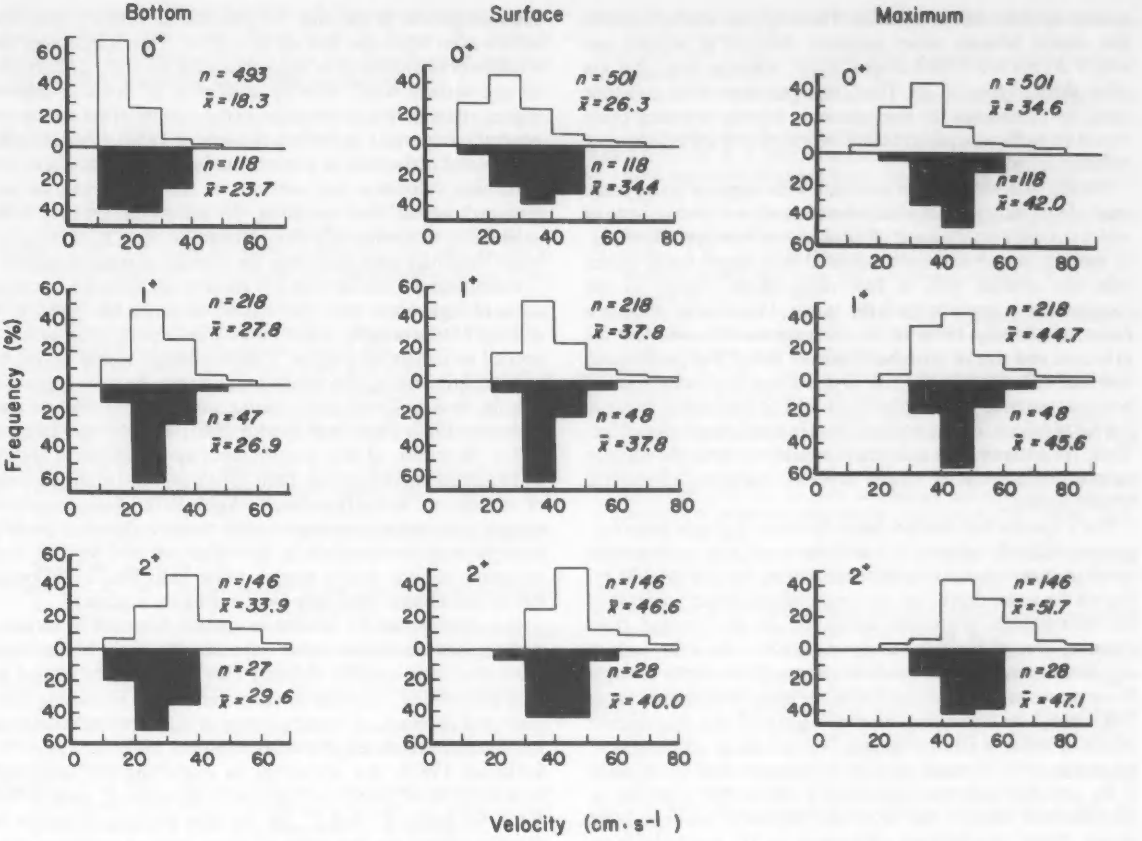

FIG. 4. Bottom water velocity, surface water velocity, and maximum water velocity within 1 m associated with positions of three age-classes of juvenile Atlantic salmon in the Little Sevogle River during summer (white) and autumn (black), 1976–78.

velocities of 10–30 cm·s^{-1} (mean = 16.8), 1^+ fish 20–40 cm·s^{-1} (mean = 29.3), and 2^+ fish 30–50 cm·s^{-1} (mean = 38.4) (Fig. 3). The focal velocities occupied by each age-class were significantly different ($P < 0.01$) between summer and autumn, being much less during the autumn. In autumn, all age-classes occurred mainly in focal velocities of $<$10 cm·s^{-1}. Fish in focal velocities $>$10 cm·s^{-1} in autumn were not sheltering beneath rocks at the time of observation.

During the summer, the bottom, surface, and maximum water velocities occupied by salmon varied significantly ($P < 0.01$) with fish age and, hence, size. Generally, these selected water velocities were about 10 cm·s^{-1} higher for each successive year of age (Fig. 4). The bottom, surface, and maximum water velocities associated with the positions of 0^+ and 2^+ fish differed significantly ($P < 0.01$) from summer to autumn when the youngest fish moved into higher velocities while the oldest fish were leaving the highest velocities occupied previously. However, there were no significant differences between summer and autumn in these properties for 1^+ fish. Despite the autumnal changes in water velocities occupied by two of the age-classes, significant age- and size-related differences ($P < 0.01$) persisted into the autumn, the velocities occupied still increasing with age.

Discussion

Rimmer et al. (1983) reported that juvenile Atlantic salmon in the Little Sevogle River did not change their habitat (riffle, run, pool) with the coming of autumn. However, they noted a rapid disappearance of salmon into the substrate in autumn. In the present study we have shown that large changes in microhabitat selection by salmon occur at this time although there is little change in their selection of habitat.

The total range of water depths occupied was much less in the autumn than in the summer. Movement away from the shallow areas in autumn removes fish from areas prone to freezing or dewatering in autumn and winter. Thus, their behavior is similar to that of coho salmon and steelhead trout (*Salmo gairdneri*), which select deeper water during autumn and winter (Hartman 1965; Bustard and Narver 1975), but dissimilar in that they occurred less frequently in deep water during the autumn. This is because juvenile coho salmon and 1^+ steelhead trout shelter about objects that are usually associated with deep pools; but 0^+ steelhead trout, like Atlantic salmon, shelter beneath stones of the streambed and do not move to deeper water as temperature falls (Bustard and Narver 1975). Probably, movement into deeper water is, to some degree, a consequence of adopting species-specific shelter material. Those species and age-classes that shelter beneath stony substrate material in autumn and winter do not move into deeper water, whereas those that use other shelter types do so. Thus, manipulation of the substrate must be considered in management. Merely creating pools would be inefficient in increasing overwintering areas for young Atlantic salmon.

During summer, all ages associate with stones of a size range most abundantly available and therefore do not discriminate or select critically on the basis of stone size at this time. However, in autumn, larger fish are associated with larger home stones than are smaller fish, a fact most likely related to the requirement of space beneath the stones. Presumably, there is a direct relationship between the dimensions of stones and the existence and size of chambers beneath them. The pronounced seasonal difference in the size distributions and mean sizes of home stones used by the salmon, as well as the marked peaks in the distributions, indicates that 20 cm is a critical substrate size. Thus, for a stream area to be most suitable for juvenile Atlantic salmon, there must be ample substrate material >20 cm for winter shelter.

For a species that shelters beneath stones during winter (e.g. juvenile Atlantic salmon, 0^+ steelhead trout), the substrate size is of primary importance in determining stream suitability. Except for water depth, the seasonal changes in the selection of all microhabitat properties depended on or resulted from attaining shelter beneath stones of suitable size. The lack of significant movement of juvenile salmon in the Little Sevogle River as water temperature fell in the autumn (Rimmer et al. 1983) could be attributable to the ample presence of substrate stones of suitable size for shelter. Movement to other areas of the stream at the summer–autumn transition would be expected if the substrate material were small (<20 cm) or if natural or man-induced siltation had filled the interstices between large stones. Thus, downstream movement of 0^+ steelhead trout during autumn and at low water temperature was reduced by the addition of large substrate material (Chapman and Bjornn 1969; Bjornn 1971). A similar effect of large stones on enhanced overwintering may have been at least partially responsible for the approximately fourfold increase in the average population densities of juvenile Atlantic salmon in areas where large stones were added to parts of the Big Tracadie River, N.B. (Gilbert 1978). A possible lack of large substrate upstream of their study areas in two small Miramichi River tributaries may have been a partial reason why Randall and Paim (1982) found that up to 53% of the 1^+ Atlantic salmon and 80% of the 2^+ salmon populations were immigrants during October.

In autumn, almost all salmon in this study were found in substrate chambers where the focal water velocity was <10 $\mathrm{cm \cdot s^{-1}}$ (54, 68, and 82% lower than in summer for 0^+, 1^+, and 2^+, respectively). Because of their rapid movement to substrate chambers, young Atlantic salmon do not display the relationship between focal water velocity and temperature reported for coho salmon that do not shelter in chambers (Bustard and Narver 1975). Movement into the places with lowest available focal water velocity, caused by a reduced ability to oppose water currents at low temperature, could be a major influence in the initiation of winter sheltering behavior of salmon at the summer–autumn transition. However, the lack of a focal velocity–temperature relationship suggests that something else, perhaps photoperiod, is also an important initiator of sheltering.

The significant age-specific differences in all of bottom, surface, and maximum water velocities selected during the summer persist in autumn, so differential velocity selection occurs even while the fish are in shelter. This emphasizes the wisdom of maintaining or augmenting the diversity of horizontal and vertical water velocity profiles at all times in salmon rearing streams. Water velocities in the vicinity of the station are generally important in feeding (Kalleberg 1958). The age- and size-related difference in bottom, surface, and particularly the maximum velocities may ensure that the largest salmon are stationed where they are likely to encounter greatest food availability whenever they emerge from shelter, i.e. near higher water velocities than those near the stations of smaller salmon.

Maintaining a station with low focal water velocity, but near areas of high water velocity, would maximize the quantity of drifting food available while minimizing energy expenditures needed to remain on station. This advantage would persist to some degree during the autumn and winter because salmonid fish do feed and may grow during cold seasons (Power and Coleman 1967; Frost and Brown 1967; Riddell and Leggett 1981). However, at low temperatures the acceleration ability and burst swimming speed, both very important in the feeding of salmon, are severely reduced (Webb 1978). Hence, a strict energy conservation strategy would be advantageous. Such a strategy may be apparent in the behavior of 2-yr-olds that occupied stations where nearby water velocities were lower during the autumn than they were during the summer.

It is notable that 0^+ salmon in autumn occurred in bottom, surface, and maximum water velocities that were higher than those near their summer stations. This could also be related to food availability. Autumn is characterized by increasing biomass and numbers of stream insects with a preponderance of small, newly recruited individuals (Hynes 1970, p. 294–299; Schlosser 1982). An advantage in exploiting the autumnal increase of small insects will accrue to the small 0^+ salmon but not to the larger 1^+ and 2^+ fish because feeding efficiency is directly related to the ratio of prey size to predator size (Paloheimo and Dickie 1966; Protosov 1970). In effect, the food availability for 0^+ salmon increases in the autumn, while that for the older fish does not. The significant changes that occurred in the selection of microhabitat properties at the summer–autumn transition indicate that all properties are important in determining the autumn and winter suitability of a stream for juvenile Atlantic salmon. The consistent independence of microhabitat properties occupied by the salmon from the microhabitat properties available (i.e. water depth, home stone size) indicates that the fish were selecting magnitudes of these properties appropriate to their seasonal needs.

To be fully successful, strategies for population enhancement must make available to salmon not just one but all of the required microhabitat components known to be selected by all age-classes in both warm and cold seasons. It appears that to enhance the autumn and winter suitability of a stream for Atlantic salmon populations composed of 0^+–2^+ age-classes, while not decreasing the summer suitability, the substrate surface should be roughened by adding large (>20 cm), unrounded stones as potential shelter where water depth in winter is 24–36 cm, where water velocities are 10–60 $\mathrm{cm \cdot s^{-1}}$, and where substrate siltation is not evident.

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Inter- and Intra-Population Variation in the Fecundity of Chinook Salmon (*Oncorhynchus tshawytscha*) and its Relevance to Life History Theory

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Healey, M. C., and W. R. Heard. 1984. Inter- and intra-population variation in the fecundity of chinook salmon (*Oncorhynchus tshawytscha*) and its relevance to life history theory. Can. J. Fish. Aquat. Sci. 41: 476–483.

Chinook salmon (*Oncorhynchus tshawytscha*) varied significantly in fecundity both between years within populations and between populations throughout their range. Generally less than 50% of the variation in fecundity between individuals within populations could be explained by variation in length. A small additional amount of variation could be attributed to racial differences (e.g. red or white fleshed types) but age, seasonal timing of subpopulations, and stream or ocean type life history pattern did not contribute significantly to variation in fecundity beyond their correlation with length. A great deal of individual variation in fecundity remains to be explained in chinook salmon. The slopes of the regression of fecundity on length for all populations were low in comparison with other fishes, indicating that fecundity increases slowly with increasing size in chinook. The mean age of reproducing females varied among populations, and populations that reproduced at an older age were more fecund at a common length than populations that reproduced at a younger age. The increase in fecundity with increasing age of maturity was consistent with theoretical predictions of the trade-off between fecundity and mortality in fish of reproductive age. The mean age of reproduction within a population, however, was considerably older than the predicted optimum age of reproduction based on the trade-off between increasing fecundity with age and natural mortality. These observations suggest that chinook have sacrificed fecundity for increased size in the allocation of surplus energy, a life history characteristic that is consistent with the survival value of size in anadromous salmon.

La fécondité du saumon chinook (*Oncorhynchus tshawytscha*) subit des variations significatives tant interannuelles au sein d'une même population qu'interpopulations dans l'aire de distribution. En général, moins de 50% de la variation de fécondité entre individus d'une même population peuvent s'expliquer par la variation de longueur. On peut attribuer à des différences raciales (p.ex. types à chair rouge ou blanche) un certain degré de variation supplémentaire, mais l'âge, le calendrier saisonnier des sous-populations et les caractéristiques du cycle de vie tant en rivière qu'en mer ne contribuent pas de façon significative à la variation de fécondité au-delà de sa corrélation avec la longueur. Chez cette espèce, la variation individuelle de fécondité est encore bien mal comprise. Comparativement à ce que l'on observe chez les autres espèces de poissons, la régression de la fécondité sur la longueur est à pente douce pour toutes les populations, signe que la fécondité augmente lentement en fonction de l'augmentation de taille du saumon chinook. L'âge moyen de la reproduction des femelles varie d'une population à l'autre, et les populations qui se reproduisent à un âge plus avancé ont, à une longueur donnée, une plus grande fécondité que les populations se reproduisant plus tôt. Une fécondité qui augmente en fonction de l'âge de maturité est en accord avec les prédictions théoriques d'un compromis entre fécondité et mortalité chez les poissons sexuellement matures. Cependant, l'âge moyen de la reproduction au sein d'une population est de beaucoup supérieur à l'âge de reproduction optimal prédit sur la base d'un compromis entre une fécondité qui augmente en fonction de l'âge et la mortalité naturelle. Ces observations donnent à penser que les saumons chinook, dans la répartition de leur surplus d'énergie, ont sacrifié la fécondité en faveur d'une croissance accrue, une caractéristique du cycle de vie en accord avec les avantages qu'offre la taille pour la survie des saumons anadromes.

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The fecundity of a species is a value of considerable practical importance to fishery managers and culturists because it is essential to estimates of seeding rate of spawning beds, to brood stock requirements, and to back-calculation of spawning stock from measurements of egg abundance. Fecundity is also of theoretical interest because energy invested in fecundity is not available for other functions such as growing, foraging, or escaping predators. How an animal invests its energy capital among various opportunities for growth, reproduction, and survival has important implications for the success of different life history patterns (Ware 1982; Stearns 1976; Bell 1980).

No comprehensive review of the fecundity of Pacific salmon has been published since Rounsefell (1957) described inter- and intra-population variation in fecundity of a number of salmonids. Only limited data were available on chinook salmon (*Oncorhynchus tshawytscha*) at that time. These data showed a large interpopulation difference between Klamath and Sacramento rivers' (California) chinook, Sacramento fish having almost twice the fecundity of Klamath fish at the same length (McGregor 1923). Chinook fecundity also appeared to increase with decreasing latitude (samples from the Strait of Georgia, the Columbia River, and California). More recently, Galbreath and Ridenhour (1964) found no significant differences among the regressions of fecundity on length for different ages or different seasonal runs of chinook to the Columbia River. The fecundity of Columbia River chinook was intermediate between that of the Klamath and Sacramento rivers, and differed significantly from both. These, plus a few reports describing fecundity as one of a number of population characteristics for particular chinook populations (Godfrey 1968; Yancey and Thorsteinson 1963; Vronskiy 1972), constitute the published record of chinook salmon fecundity. Considerable additional unpublished material on fecundity and other parameters of chinook populations in British Columbia and Alaska has been made available to us. These data permit an extensive empirical description of inter- and intra-population variation in fecundity of chinook salmon and also provide insights into their reproductive strategy.

Both Stearns (1976, 1977) and Bell (1980) commented on the paucity of empirical tests of the predictions of life history theory. One important reason for this is the difficulty in obtaining sufficiently reliable data over a range of life history types, or environments, within a single species against which to test predictions. Among the more successful attempts to relate theory and empirical observation were those of Schaffer and Elson (1975) for Atlantic Salmon (*Salmo salar*) and Leggett and Carscadden (1978) for American shad (*Alosa sapidissima*). In both these studies a wide-ranging anadromous species with variable life history provided an opportunity to examine some of the predictions of life history theory. The chinook salmon is a Pacific species with similar characteristics. The geographic distribution of chinook extends from Kamchatka around the North Pacific to central California. Chinook are anadromous, and their spawning beds are located from a few to over 1000 km upstream. Juvenile chinook migrate to sea in their first to third years and females mature in their third to seventh years. Maturing fish may enter the spawning streams almost any time of year, but there are commonly spring, summer, and autumn runs. Thus, chinook present an opportunity to evaluate some of the predictions of life history theory similar to that presented by American shad or Atlantic salmon.

The purpose of this paper, therefore, is twofold. First, we describe inter- and intra-population variation in fecundity and some other parameters of reproduction in chinook salmon. Second, we relate this variation to predictions of life history theory in a way analogous to that of Schaffer and Elson (1975) and Leggett and Carscadden (1978).

Materials and Methods

Both published and unpublished data, representing virtually the whole geographic range of chinook (Fig. 1), form the empirical basis of this report.

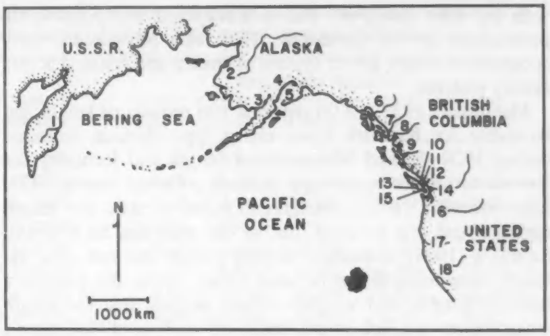

FIG. 1. North Pacific and Bering Sea regions showing the locations of the chinook populations referred to in the text: 1, Kamchatka; 2, Yukon; 3, Nushagak; 4, Cook Inlet; 5, Kenai; 6, Taku; 7, Nass; 8, Skeena; 9, Rivers Inlet; 10, Quinsam; 11, Puntledge; 12, Big Qualicum; 13, Somass; 14, Fraser; 15, Nitinat; 16, Columbia; 17, Klamath; 18, Sacramento.

Published Data

Vronskiy (1972) reported 59 records of length and fecundity for chinook of the Kamchatka River, USSR, collected during 1965–69, as well as information on age and sex composition of the spawning run. Unfortunately, Vronskiy neglected to state the length measurement employed in his study, so that these data can only be compared in a general way with data from North America. Nor did he present separate estimates of age composition for males and females. The Kamchatka data were from a mixture of spawning populations, most of which were presumably stream type fish (chinook that go to sea after a year or more in freshwater).

Yancey and Thorsteinson (1963) reported 60 records of length, weight, and fecundity for chinook from Cook Inlet, Alaska, collected during 1959, and information on the age composition of the population. These data represented a large number of spawning populations in rivers tributary to Cook Inlet. Virtually all fish sampled were stream type.

Godfrey (1968) reported coefficients for the regression of log fecundity on log length for chinook from the Nass, Skeena, and Fraser rivers, British Columbia, collected during 1964–66, as well as the age composition of the populations. These data were from samples taken near the river mouths and thus represent a mixture of the numerous spawning populations of chinook in each river. The statistics permit comparison among these rivers and other North American rivers, but because the individual lengths and fecundities were not reported, these populations could not be included in an overall analysis of fecundity.

Godfrey's (1968) data provide a comparison of fecundity among red and white flesh colors within the Fraser River. Both stream and ocean type (chinook that migrate to sea during their first year of life) chinook were represented in the samples, but Godfrey (1968) did not analyze the data from these two life history types separately.

Galbreath and Ridenhour (1964) reported 62 records of length, age, and fecundity for Columbia River chinook collected during 1959. These data were analyzed by Galbreath and Ridenhour without transformation. We have reanalyzed their data using $\log_e$ transformed variables to permit comparison with our other analyses. The data represent many spawning populations in the Columbia. They also provide the only comparison within a river system of stream and ocean type life history patterns.

McGregor (1922, 1923) reported 106 records of length and fecundity for Klamath River ocean type chinook collected during 1920–22 and 50 records of length and fecundity for Sacramento River ocean type chinook collected during 1922. Like Vronskiy (1972), McGregor failed to state the length measurement that he used, but he did state that he followed Snyder's (1921) technique. Snyder (1921) did not state the length measurement that he used either, but he did provide a table of lengths and weights. These suggest that the length measurement was fork length, and in our analysis of McGregor's data we have assumed that the lengths given were fork lengths.

Unpublished Data

Previously unpublished data that we analyze in this report include 15 records of average length, age, and fecundity of Yukon River stream type chinook collected by the Alaska Department of Fish and Game (ADFG) (Geiger 1965; Bigler 1981) during 1965 and 1981. These data are from chinook taken in river fisheries and represent a mixture of spawning populations. The total number of females collected was 114, but fecundity was averaged by age and date of capture, giving a total of 15 separate records. Also made available to us were 31 records of length, age, and fecundity for Nushagak River stream type chinook collected by ADFG during 1966 and 1968 (Nelson 1969).

Sixty-three records of length and fecundity of Kenai River stream type chinook and 33 records of Taku River stream type chinook were collected by one of us (W.R.H.) in 1981. The Kenai River data are from early and late run fish captured in the river. These fish differ in size and spawning location. The Kenai River data also represent a single Cook Inlet tributary and may be compared with the mixed sample reported by Yancey and Thorsteinson (1963). The Taku River data are from chinook captured in the river mouth fishery and may represent several spawning populations in the river.

Data on length, fecundity, and other population characteristics collected by staff of the Departmenrt of Fisheries and Oceans and made available to us include 24 records of length and fecundity for Rivers Inlet (Wannock River) ocean type chinook collected during 1973 from samples taken in the river mouth sport fishery (these data are probably representative of a single spawning population), 26 records of length and fecundity for Puntledge River ocean type chinook collected during egg taking for the Puntledge River hatchery in 1973, 1975, and 1976 (these data represent an unusual local summer run of ocean type chinook), 14 records of length and fecundity for Nitinat River ocean type chinook collected during feasibility studies for the Nitinat River hatchery in 1979 (these data represent the single spawning population in the river), 583 records of length, age, and fecundity for Big Qualicum River ocean type chinook collected during egg taking for the Big Qualicum hatchery in 1960, 1962, and 1968–73 (these data represent the single spawning population in the river), 178 records of length, age, and fecundity for Quinsam (Campbell) River ocean type chinook collected during egg taking for the Quinsam hatchery in 1974–77 (these data represent the single spawning population in the Campbell River), and 19 records of length and fecundity for Robertson Creek (Somass River) ocean type chinook collected during egg taking for the Robertson creek hatchery in 1976 (these data represent the single spawning population in the Somass River).

Analytical Methods

The methods for determining fecundity ranged from total counts (Yukon, Cook Inlet, Kenai, Taku, and Columbia River data) to a variety of gravimetric subsampling methods (all others except Kamchatka). Vronskiy (1972) did not state the method he used for estimating the fecundity of Kamchatka River chinook.

Several different measurements of length occur in the data sets, including length from the mideye to the fork of the tail (MEF) (most Alaskan data), length from the posterior margin of the orbit to the end of the hypural plate (POH) (all B.C. data), and length from the snout to the fork of the tail (FL) (Columbia, Klamath, and Sacramento rivers data). The length measurement used throughout this report is POH. We used the following formulae to convert MEF and FL to POH (all lengths in millimetres):

$$\mathrm{POH} = 0.859\mathrm{MEF} + 25.7$$

$$\mathrm{POH} = 0.761\mathrm{FL} + 47.2.$$

We derived these formulae from measurements made on mixed groups of chinook from Alaska and British Columbia. The total sample size was 155 and the fish ranged in length (POH) from 401 to 987 mm.

Age composition data for the populations analyzed in this report were taken either from the publication that provided the fecundity data or other published material on the population or was included with the raw data on fecundity and length provided to us. Most of the age composition data were from samples taken by netting, and the ages were all determined from scales.

The principal statistical analysis that we employed for comparing fecundity within and between populations was analysis of covariance with length as a covariate. In all instances we transformed length and fecundity values to natural logarithms before analysis. Previous analyses of Pacific salmon fecundity have been on untransformed variables (Rounsefell 1957; Galbreath and Ridenhour 1964), the argument being that transformation did not significantly increase the amount of variation explained by the regression analysis. Theoretically, however, fecundity should be approximately proportional to the cube of length. The logarithmic transformation is, therefore, appropriate to make the relationship linear and stabilize variances. Even though some authors have performed analysis of variance on their data, we have repeated all analyses using transformed variables to permit comparison among populations.

To separate the effects of age and length in the analysis of covariance, we regarded ages as treatments and compared adjusted mean fecundity among ages in the analysis of covariance.

Results

Intrapopulation Variation in Fecundity

We expected a significant positive correlation between $\log_e$ fecundity and $\log_e$ length and in all instances, except for the small sample from Nitinat River, the correlation was significant (Table 1). At best, however, variation in length explained only 76% of variation in fecundity and in most instances (11/15) explained less than 50%. The large residual variation is not easy

TABLE 1. Geometric mean postorbit–hypural length, geometric mean fecundity, range of length and fecundity, and regression coefficients for the regression of $\log_e$ fecundity on $\log_e$ length for 18 chinook populations (data sources are noted in the text).

Population	Sample size	$\tilde{X}$ length	Range of length	$\tilde{X}$ fecundity	Range of fecundity	Regression statistics[a]				
						a	b	SD b	r	F
Sacramento	50	737	474–884	7295	4795–11 012	4.94	0.599	0.210	0.381	8.14
Klamath	106	694	442–860	3634	1718–8406	−0.74	1.380	0.148	0.676	87.75
Columbia	62	684	492–840	4916	2148–7705	−2.95	1.754	0.128	0.870	187.10
Fraser[b]	351	837	580–1070	5800	2638–8869	−0.61	1.412			
Nitinat	14	759	665–850	4991	3653–6290	0.12	1.264	0.652	0.491	3.76
Robertson	19	720	575–853	4452	2943–6799	1.47	1.053	0.351	0.588	8.98
Qualicum	583	746	570–895	4982	1622–7953	0.42	1.193	0.274	0.409	111.20
Puntledge	26	710	620–875	4604	2763–7306	−6.17	2.220	0.459	0.703	20.51
Quinsam	178	864	713–1001	6720	2389–11 640	−4.53	1.973	0.274	0.476	51.59
Rivers Inlet	24	941	670–1090	9790	7200–12 550	3.79	0.788	0.187	0.669	17.83
Skenna[b]	175	931	427–1330	6789	2058–11 537	−5.87	2.208			
Nass[b]	43	931	627–1082	6531	4713–9513	0.60	1.231			
Taku	33	742	637–845	5504	4041–7848	−0.92	1.442	0.369	0.574	15.25
Kenai	63	844	683–987	10 622	6521–17 255	−3.77	1.935	0.209	0.764	85.76
Cook Inlet	60	754	599–889	8341	4242–13 619	−2.78	1.782	0.181	0.790	96.40
Nushagak	69	783	635–936	10 137	5302–16 049	0.49	1.311	0.277	0.508	23.35
Yukon	15	753	600–815	8668	5821–11 278	−1.93	1.660	0.656	0.575	6.41
Kamchatka	31	900	725–1125	9191	5000–15 000	−2.40	1.695	0.164	0.808	107.30

[a] a = intercept, b = slope, SD b = standard deviation of slope, r = correlation coefficient, F = F statistic for the correlation coefficient (all values significant at $P < 0.05$ or less except that for Nitinat).

[b] Means of length and fecundity are arithmetic rather than geometric.

to account for. Variation in age within samples made no contribution to variation in fecundity beyond that predicted by differences in length among ages (8 populations, F value range 0.02–2.9, all $P > 0.05$). Comparisons between seasonal runs in the Kenai River ($F = 3.71$, df 1/60, $P > 0.05$) and also in the Columbia River ($F = 1.65$, df 3/57, $P > 0.05$) were not significant, nor was there any difference between stream and ocean type life histories in the Columbia ($F = 1.0$, df 1/60, $P > 0.05$). Godfrey (1968), however, observed a significant difference in adjusted treatment means between red- and white-fleshed chinook in the Fraser River, red-fleshed fish having the higher fecundity. In four of the seven populations for which we have more than 1 yr of data, we found variation between years to be significant (Table 2). Godfrey (1968) also observed significant variation in fecundity between years in the Fraser River. The differences between years are not large, however, and when annual variation is taken into account the variation in fecundity that was explained only increased from 34 to 45% in the four populations. Clearly, a great deal of variation in fecundity within populations remains to be explained.

Interpopulation Variation in Fecundity

Differences in fecundity among populations of a species are a rather common observation (Bagenal 1978). The average fecundity of chinook varied threefold among the samples we analyzed, from 3634 for the Klamath River to 10 622 for the Kenai River (Table 1). The average length of females also varied considerably among samples, however, so that at least part of the intersample variation in fecundity is due to variation in length. The slopes of the regressions of fecundity on length varied more than threefold among populations (0.599–2.220, Table 1), suggesting that populations might differ in both the elevation and slope of the regression of fecundity on length. We tested for differences in slope among populations but excluded the Nass, Skeena, and Fraser rivers from this analysis because data on individual lengths and fecundities were not available, and we excluded Kamchatka River because of uncertainty about the length measurement. Differences in slope among the remaining 14 populations were significant ($F = 3.05$, df 12/1274, $P < 0.01$). Four populations contributed to this significant difference: the Sacramento and Rivers Inlet populations, which had low slopes, and the Puntledge and Quinsam populations, which had high slopes. Fish from the Sacramento, Quinsam, and Rivers Inlet populations were also large in comparison with adjacent populations and had high fecundity. Puntledge chinook were not large, but, for the geographic area, represent an unusual summer run. Although the average fecundity of these populations cannot be compared directly with that of the other populations because of the differences in slope, these populations may, for the same reason, be regarded as having unique fecundities.

Among the remaining 10 populations that had a common slope, differences in elevation were significant ($F = 157.9$, df 9/1013, $P < 0.001$). Mean fecundities adjusted to 740 mm POH ranged from 4543 to 8861, almost a twofold difference (Table 3).

Comparison of the fecundity of populations from different latitudes suggests that, if there is a trend with latitude, it is toward higher fecundity at higher latitude (Table 3) rather than the reverse as Rounsefell (1957) suggested. All of the populations from Rivers Inlet north, with the exception of the Taku, have fecundity over 6000 at 740 mm POH. South of Rivers Inlet all but two of the populations have fecundity less than 6000. For the 10 populations having a common slope of the regression of fecundity on length the trend is even more obvious. Populations to the north of the Taku have fecundity greater than 7000 at 740 mm POH, while those to the south have fecundity less than 6000 (Table 3). Even within these groupings, however, there are differences. Among the high-fecundity populations to the north the Nushagak chinook are significantly more fecund than the Cook Inlet or Kenai populations ($t = 3.17$ and 4.04, $P <$

TABLE 2. Variation in fecundity between years within populations. Fecundity is adjusted to common length for each population by analysis of covariance. *F* and *P* values are for analysis of covariance testing the null hypothesis of no difference between years. NS = not significant.

Population	Variable	Yearly statistics								*F*	*P*
Klamath	Year	1920	1921	1922							
	n	30	65	11							
	Eggs	3573	3676	3592						1.0	NS
Big Qualicum	Year	1960	1962	1968	1969	1970	1971	1972	1973		
	n	3	9	31	28	155	92	184	81		
	Eggs	4692	4900	4860	5215	4549	5498	5113	5223	9.27	<0.001
Puntledge	Year	1973	1975	1976							
	n	3	11	12							
	Eggs	4512	4691	4918						1.00	NS
Quinsam	Year	1974	1975	1976	1977						
	n	58	49	50	21						
	Eggs	6703	7179	6957	5940					4.46	<0.01
Nushagak	Year	1966	1968								
	n	31	38								
	Eggs	10 084	10 195							1.00	NS
Yukon	Year	1965	1981								
	n	5	10								
	Eggs	7621	9240							4.84	<0.05
Kamchatka	Year	1965	1966	1967	1968	1969					
	n	10	14	13	10	12					
	Eggs	10 348	8268	9314	8907	9533				6.14	<0.001

0.05), while among the low-fecundity populations to the south, the Taku and Columbia river populations are significantly more fecund than the other four populations (*t* value range 2.56–7.61 for 8 comparisons, all $P < 0.05$). The suggestion of a latitudinal trend in fecundity may be spurious, however, as, with the exception of the Klamath River population, all the populations with low fecundity are from river systems on Vancouver Island (Fig. 1). If Vancouver Island populations are not considered, the trend is still to increasing fecundity with latitude, but the relationship is not significant ($F = 3.76$, df 1/10, $P > 0.05$).

Ten of the 18 samples we analyzed represent a mixture of local spawning populations (see Materials and Methods). Since there is significant interpopulation variation in fecundity, some of the high residual variation in regressions of fecundity on length may be a consequence of interpopulation variation of these samples. The residual variation in samples involving a mixture of populations, however, is not significantly greater than in samples from a single local population so that the contribution of interpopulation variation to total variation in these mixed samples must be small. Comparison of the Kenai and Cook Inlet samples exemplifies this similarity among local populations. The Cook Inlet sample represents a mixture of numerous local stocks including the Kenai River, whereas the Kenai River sample includes two stocks having different run timing within the same river. There were no significant differences among any of these samples and no evidence for increasing residual variation as the number of local populations in the sample increased.

Variation in Other Population Parameters

The foregoing analysis demonstrates considerable variation in fecundity between populations. If we presume that this variation is adaptive then it must bear some relationship to population specific strategies for reproduction and survival. We shall consider three other population characteristics that are important to an interpretation of population-specific fecundities as adaptations for survival: (1) the mortality rate of chinook of reproductive age and of chinook prior to the age of first reproduction, (2) the average age of reproducing females, and (3) the variance in age of reproduction.

The mortality of chinook prior to the earliest age of reproduction has not been measured precisely for any natural population. Mortality during this period is known to be high, however, and is believed to be both variable and negatively correlated with size (Ricker 1976). The freshwater habitats of chinook are also believed to be less predictable than the marine habitats so that survival in freshwater is more uncertain. Stream type chinook, by definition, spend longer in freshwater before going to sea, grow more slowly during their first year of life, and first mature at an older total age than do ocean type chinook. Thus, stream type chinook should suffer greater and more variable mortality before reaching reproductive age than ocean type.

Parker and Kirkness (1956) estimated the mortality of chinook reproductive age in the ocean to be 0.417 (annual instantaneous rate). Both Parker (1962) and Ricker (1976) considered this value to be too high, and Parker (1962) suggested a common ocean mortality rate of 0.316 for all Pacific salmon. Most published estimates of ocean mortality are for sockeye salmon (*Oncorhynchus nerka*) and these estimates average 0.180 (Ricker 1976). As a preliminary estimate, therefore, we may conclude that the instantaneous annual mortality rate of chinook of reproductive age is in the range 0.180–0.316. Again we would anticipate that stream type chinook would have a mortality rate higher than ocean type owing to their long upriver migrations and because they return

Table 3. Approximate north latitude of natal river mouth, fecundity at 740 mm post-orbit–hypural length calculated from individual regressions of fecundity on length and, for 10 populations whose regression slopes did not differ significantly, calculated from common regression of fecundity on length, and mean total age and variance of age of females in 10 spawning populations.

Population	°N latitude	Fecundity at 740 mm Individual regression	 Common regression	Female age Mean	 Variance
Sacramento	37°45′	7313			
Klamath	41°30′	4347	4961		
Columbia, stream	46°15′	5932	6298	4.45	0.2954
Columbia, ocean	46°15′	5453	5515	4.35	0.3744
Nitinat	48°15′	4773	4543		
Fraser	49°15′	6116		4.21	0.3312
Robertson	49°15′	4568	4755		
Big Qualicum	49°25′	4031	4883	3.94	0.1487
Puntledge	49°30′	4899			
Quinsam	50°00′	4939		4.83	0.4215
Rivers Inlet	51°40′	8071			
Skeena	54°15′	6108		4.47	0.3589
Nass	55°00′	6203		4.46	0.2551
Taku	58°30′	5469	5472	5.76	0.2611
Kenai	60°15′	8216	7747	5.88	0.1782
Cook Inlet	60°00′	8047	7963	5.68	0.2560
Nushagak	58°30′	9427	8861	6.04	0.3363
Yukon	62°30′	8409	8315	5.99	0.1077
Kamchatka	56°00′	6623			

to freshwater some weeks or even months prior to spawning (Healey 1983).

The average age of reproducing females varied from 3.94 yr for Big Qualicum to 6.04 yr for Nushagak (Table 3). The ocean age of reproducing females varied less because the populations having the oldest total age were all stream type chinook that spent a year or more in freshwater before going to sea. The variance in age of reproducing females ranged from 0.1077 for the Yukon River to 0.4215 for the Quinsam (Table 3).

Relationship with Life History Theory

The average total age of reproducing females was positively correlated with average fecundity at 740 mm POH ($r = 0.776$, $P < 0.01$). According to theory, a greater age of maturity should be favored only if the instantaneous rate of increase in fecundity with age is equal to or greater than the instantaneous annual mortality of reproductively aged females (Bell 1980). The slope of the regression of $\log_e$ fecundity on average age of reproducing females is 0.243, well within the range of possible rates of mortality for chinook.

The average age of reproducing females within a population should also be a function of the age-specific fecundity of the population and the mortality of reproductively aged females that have not spawned. The instantaneous rate of change in fecundity between ages within a population is normally a decreasing function of age. Within a population the optimum age of maturity for females is the age at which the instantaneous rate of change in fecundity equals the instantaneous mortality rate of reproductively aged females (Bell 1980). Pacific salmon are particularly suitable for examining this relationship because each successive spawning age-class is unequivocally composed of virgin fish. Five of the chinook populations provide sufficient data to test this prediction. Curves for the five populations of $\log_e$ fecundity against age, all normalized to a common first age of reproduction and a common feundity at first age of reproduction, show a wide scatter (Fig. 2). For two populations the average fecundity of the oldest spawners was less than the fecundity of the preceeding age, and in one instance was less than the fecundity of the youngest spawners (Yukon River). In this instance the drop in fecundity at the oldest age was also statistically significant ($t = 3.13$, df = 10, $P < 0.05$). This result is quite contrary to theory, since for there to be any advantage to maturing at an older age, fecundity must at least increase sufficiently to balance natural mortality. Although natural mortality may well decline with age in chinook, it cannot be negative.

For all five populations the slopes of the relationship between fecundity and age are smaller than expected. In only three of the populations is the slope greater than the minimum estimate of natural mortality at any age above the earliest age of reproduction. Except for the Nushagak population, the slopes of the curves at the average age of reproduction for the population are considerably less than the minimum estimated natural mortality rate of reproductively aged chinook. If the age of maturity for these populations is linked with age-specific fecundity in the manner predicted by life history theory, then we must drastically reduce our estimate of natural mortality for chinook. If we take the approximate slope of the fecundity on age curve at the mean age of reproduction as an estimate of the natural mortality of reproductively aged chinook, then mortality ranges from −0.042 to 0.183 and averages 0.064 (or 0.073 if we set the impossible negative value for Yukon chinook to 0.00).

The curvature of the relationship between $\log_e$ fecundity and age also varies among populations from strongly curved such as for the Big Qualicum and Yukon River populations to weakly curved such as for the Nushagak and Quinsam populations (Fig. 2). This too should be related to age of maturity, since in strongly curved relationships the optimum age should be

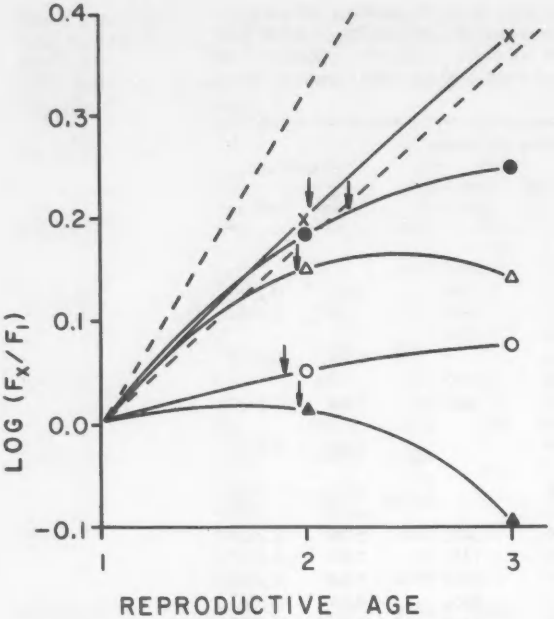

FIG. 2. Relationship between fecundity and age for Columbia River (●), Quinsam River (○), Big Qualicum (△), Nushagak River (×), and Yukon River (▲) chinook salmon. F_x/F_1 is the ratio of fecundity at reproductive age x to the fecundity at reproductive age 1 where age 1 is the earliest age of reproduction. Arrows indicate the mean reproductive age for each population. The broken lines are upper and lower estimates of the natural mortality rate.

strongly selected whereas in weakly curved relationships the optimum should be weakly selected. This occurs because in strongly curved relationships the loss in reproductive power increases very rapidly on either side of the optimum age whereas in weakly curved relationships the loss in reproductive power increases only slowly on either side of the optimum age. Thus, when the relationship is strongly curved a great deal is lost by maturing at an age other than the optimum, while in weakly curved relationships this is not the case. Therefore, we expect to find a negative correlation between the strength of curvature of the fecundity on age relationship and the variance in age of maturity in the population. Our data are consistent with this prediction, as the Big Qualicum and Yukon populations have strongly curved fecundity on age relationships and low variance in age of maturity whereas the Quinsam, Nushagak, and Columbia populations have weakly curved fecundity on age relationships and high variance in age of maturity (Table 3).

Variance in age of maturity should also be positively correlated with variance in prereproductive mortality (Stearns 1976; Schaffer and Elson 1975). One would expect, therefore, that stream type chinook would show greater variance in age of maturity than ocean type chinook. The trend in the data is in the reverse direction, however, with stream type chinook having relatively low variance in age of maturity ($S^2 = 0.108$–0.295) compared with ocean type chinook ($S^2 = 0.149$–0.442) (Table 3). The difference between life history types is not statistically significant, however.

Discussion

Several recent publications testify to a considerable variation in life history patterns in chinook (Reimers 1971; Healey 1980, 1983). The high inter- and intra-population variation in fecundity we described is, therefore, consistent with variation in other population characteristics of the species and also with variation in fecundity of other species (Bagenal 1967, 1978). Variation in fecundity may be attributed to variation in population density or feeding conditions (Dahlgren 1979; Wooton 1973) or to variation in selection gradients between populations (Stearns 1977). Chinook populations have been reduced by fishing throughout their range (e.g. Healey 1982a) so that density effects are likely to be minimal. Of the two remaining "causes" of fecundity variation, food supply appears to be most likely to affect intrapopulation variation and selection gradients to affect interpopulation variation.

Variation in fecundity between years within populations was relatively small, suggesting that average feeding conditions do not vary greatly from year to year. A perplexing feature of intrapopulation variation in fecundity, however, is the high residual variation in regressions of fecundity on length. If we presume that variation between individuals, like variation between years, is mainly a function of nutrition, then we must postulate rather large differences in foraging success among individuals. An unresolved question, however, is the relationship between fecundity and egg size in chinook. Individuals with a high fecundity relative to others of the same body size may be trading egg size for numbers, a strategy that would have interesting implications for population fitness. Unfortunately, the only data on egg size in chinook are a few measurements reported in Rounsefell (1957).

Variation between populations involved both the slope and the elevation of fecundity on length regressions. By comparison with other species, which generally have slopes in the range 3–5 (Bagenal 1967), the slopes for all chinook populations are low (0.6–2.2). Thus fecundity increases less with increasing size in chinook than it does in other fishes, and in chinook the increase is less than proportional to weight, while in other fishes it is generally more than proportional to weight. A possible explanation for the low slopes is that small chinook produce a disproportionately large number of (small) eggs. Small fish commonly produce smaller eggs than large fish from the same population (Bagenal 1967). On the other hand, large chinook may, because of their greater maintenance requirements, have less surplus energy to devote to egg production if their food resources are limiting. Large fish may, in fact, have sacrificed egg production for body size in the disposition of surplus energy, since size has both survival value and reproductive value in Pacific salmon.

The significant differences in slope among populations could conceivably be a result of differences in foraging conditions encountered by different populations in the ocean. This appears to be an unlikely explanation given the intermingling of chinook populations in the ocean and the close geographic proximity of populations with different slopes (e.g. Big Qualicum and Puntledge). More likely, the differences in slope reflect different relative advantages of egg number, egg size, and body size in different river systems, as suggested by Rounsefell (1957).

As with variation in slope, variation in elevation of the fecundity on length relationship could reflect differences in nutrition among populations, but this is again an improbable explanation. It is more likely that the differences in elevation reflect local adaptations to spawning and rearing conditions. Most of the high-fecundity populations, for example, are stream type chinook and for these populations high fecundity may be necessary to offset high prereproductive mortality and older age of maturity. Schaffer and Elson (1975) and Leggett and Carscadden (1978) found analogous relationships in Atlantic salmon and American shad although the fact that these species are partially iteroparous adds an additional dimension to the problem and complicates direct comparison with chinook.

According to life history theory, age-specific patterns of mortality, maturity, and fecundity are interrelated in specific ways, although there is considerable ambiguity in the theory (Stearns 1977). The pattern of mortality, maturity, and fecundity observed in chinook is not wholly consistent with predictions of the theory. In particular, chinook appear to delay maturation beyond the optimum age suggested by their ocean mortality rate and changes in fecundity with age (Bell 1980). While this result is clearly contrary to a theoretical prediction, it cannot be considered a critical test of the theory. Our data, as with most field data, meet only a few of the criteria Stearns (1977) proposed for a critical test of life history theory. Results such as ours cannot be simply dismissed, however, for, as Stearns (1977) observed, about half of the best published studies do not agree with theory. Our observations appear to be in good company in this regard.

A possible explanation for our contrary result lies in the trade-off between body size and fecundity. Earlier we suggested that size had both survival value and reproductive value in Pacific salmon. The survival value of size has been demonstrated for small salmon (e.g. Healey 1982b) and is presumed to exist for larger salmon as well (Mathews and Buckley 1976). The reproductive value of size stems from two factors. First, Pacific salmon undertake a long-distance spawning migration, much of it against a considerable current velocity. The spawning site is on a riffle, and the female must hold position on the riffle, dig a spawning nest, and protect it from conspecifics and predators. During most of this energy-demanding process Pacific salmon do not eat. Large body size and abundant energy reserves must, in most instances, confer an advantage in this situation (see Schaffer and Elson 1975). Chinook, in particular, will spawn in deeper, swifter water than the other species and in coarser gravel (Bruner 1951). Second, spawning females are territorial. Large size must confer a social advantage, and the larger females should obtain the best spawning sites and attract the best males. Thus, maternal body size may confer sufficient survival advantage to the progeny that the disadvantages of lower relative fecundity are overcome.

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Population Dynamics of Western Arctic Bowhead Whales (*Balaena mysticetus*)

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Leslie-type projection matrices were used to model the western Arctic bowhead whale (*Balaena mysticetus*) population in two stages: (1) from 1848 to 1970 and (2) from 1970 to 2000. Several constraints were imposed on adult and immature survival rates, and several assumptions were made about combinations of age through which immature survival applies (*a*), age at sexual maturity (*m*), and maximum age (*w*). Fecundity was derived by assuming that the population was stationary in 1848 (start of commercial exploitation). Estimates of numbers of animals killed and struck and lost, as well as an assumed mortality rate for whales struck and lost, were used to solve for the 1848 population size assuming that the 1970 level was 4000 bowheads. The 1970 level (4000) was then projected forward to the year 2000 with constant kill regimes for 1983–2000 of 0, 20, and 40 animals per year. Results indicate that there is an apparent incompatibility between the observed proportion of immature whales in the population and the proportions predicted by population modeling. Improved estimates of calf production and of the proportion of immature bowheads in the population are therefore needed. Calculated maximum net productivity rate is very sensitive to adult survival rate and thus, accurate estimates of the current growth rate of the bowhead whale population are not possible at present. Due to the many uncertainties about relevant biological features of the population, it would be prudent to restrict future total removals to no greater than the levels prior to 1970.

Des matrices de projection de type Leslie ont été utilisées dans l'élaboration d'un modèle de la population de baleines franches (*Balaena mysticetus*) de l'Arctique occidental, en deux phases : (1) de 1848 à 1970 et (2) de 1970 à 2000. On a imposé plusieurs contraintes aux taux de survie des adultes et des immatures, et formulé plusieurs hypothèses sur les combinaisons d'âges auxquelles s'appliquent la survie des immatures (*a*), l'âge de maturité sexuelle (*m*) et l'âge maximal (*w*). La fécondité a été estimée en supposant que la population était stationnaire en 1848 (début de l'exploitation commerciale). L'effectif de la population en 1848 a été établi à partir d'estimations des nombres d'animaux tués ou encore atteints et perdus, ainsi que d'un taux de mortalité supposé de baleines atteintes et perdues et, enfin, supposant que le niveau de 1970 était de 4000 baleines franches. Ce niveau de 1970 (4000) a été ensuite projeté à l'an 2000 à des régimes constants d'animaux tués de 1983 à 2000 de 0, 20 et 40 baleines par année. Les résultats mettent en évidence une contradiction apparente entre la proportion de baleines immatures observées dans la population et les proportions prédites par modélisation de la population. On a donc besoin de meilleures estimations de production de veaux et de proportion de baleines franches immatures dans la population. Le taux de productivité nette maximale calculé est très sensible au taux de survie des adultes ; c'est pourquoi il est actuellement impossible d'estimer de façon précise le taux de croissance présent de la population de baleines franches. A cause des nombreuses incertitudes qui entourent les caractéristiques biologiques pertinentes de cette population, il serait prudent de limiter la récolte totale future à des niveaux ne dépassant pas ceux d'avant 1970.

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At each of its meetings from 1977 to 1982, the Scientific Committee of the International Whaling Commission (IWC) recommended that no bowhead whales (*Balaena mysticetus*) be removed from the western Arctic population (the stock inhabiting the Bering, Chukchi, and Beaufort seas) (IWC 1978, 1982). Since the bowhead whale take is part of the Alaska Eskimo subsistence hunt the underlying effect of this recommendation, had it been implemented, would have been the removal of the exemption in the IWC Schedule of Regulations for aboriginal whaling. The reasons for this recommendation were an increasing kill of bowheads, a suspected low population size, and a lack of adequate population and life history data (Tillman 1980). The lack of adequate biological data, and especially the low apparent gross recruitment rate, constitute continuing problems in judging the impact on the bowhead population by the Alaskan Eskimo harvest. An additional concern is the potential effects of Outer Continental Shelf oil and gas development on the bowhead population.

Subsequent to the 1977 IWC meeting, the U.S. National Marine Fisheries Service developed and implemented a comprehensive research and management program (Braham et al. 1979). Initial results of the research indicated that the population might be in the range of 1700–2900 individuals (Braham et al. 1979; Krogman 1980). Subsequent improvements in the analysis of the census data suggested a recent population level of about 3800 whales (IWC 1983). The current estimate is 10–44% of the previously reported estimates of the initial level in 1848 when commercial whaling began, which range from 9000 to 40 000 (Mitchell 1977; Breiwick et al. 1980; Bockstoce and Botkin 1983; Breiwick and Mitchell 1983; Tillman et al. 1983). The present study indicates that the 1848 population was in the range of about 14 000–20 000 animals.

The western Arctic bowhead whale population sustained a limited Eskimo harvest for several thousand years before yankee whaling, which began in 1848 and terminated about 1914 (Marquette and Bockstoce 1980; Breiwick and Mitchell 1983). From 1914 to 1970 an average of about 12 whales per year were landed by Alaskan Eskimos (Marquette 1979; Tillman 1980). Although the effects of the 1914–70 harvest on recovery of the population are not known, Eberhardt and Breiwick (1980) suggested some reasons for believing that the population may have increased under this reduced exploitation. After 1970, however, the number of whales landed increased about threefold to approximately 30 whales per year, with an increase also in whales struck and lost — from an estimated total kill (assuming 100% mortality of struck and lost whales) of 33 in 1969 to 111 in 1977 (Johnson et al. 1981; Marquette et al. 1982; Breiwick and Mitchell 1983).

A significant increase in the rate of removal on an already depleted population indicated a need to be concerned about possible extinction. This concern led to a very preliminary evaluation by IWC scientists, in 1979, of the limited data on recruitment, removals, and harvest composition. They concluded that "... a very high proportion of the recruitment to the adult stock has been removed ..." and "... in which case the population will decline" (IWC 1980, p. 104). These conclusions of the Subcommittee on Protected Species and Aboriginal Whaling were based on a projection model in which animals killed in the Eskimo harvest were removed from the survivors of the previous year, beginning in 1973 and running through 1979. The assumptions used to make this evaluation were (1) 50% of the struck and lost whales died, (2) the sex and age distributions of whales landed as well as struck and lost were the same, (3) a "best" estimate of gross recruitment of 2.5% (based on minimum calf counts), (4) bowheads reach sexual maturity at age 6 or about 15.2 m in length, (5) immature bowhead age-classes occur in approximately 1.5-m length intervals from age 1 (7.6 m) to age 6 (15.2 m), (6) 90% of the harvest was from the immature component of the population, and (7) juvenile mortality is 10% per year. The results of their analyses indicated that the harvest level and population structure from 1973 through 1978 prevented any net recruitment to the adult population (IWC 1980). Since the time of that analysis, some length-at-maturity information has been obtained, as well as new data on the size distribution of the catch, population size, and mortality of those whales struck and lost. We now believe that a more reliable analysis is possible.

The purpose of this paper is to assess the risk to the bowhead population from past and future harvests, and particularly for the period of increasing takes from 1970 to 1977, and to evaluate the sensitivity of population projections to various life history parameters and harvest strategies. Because some important information is still lacking, and much of the information on life history parameters and harvest data comes from a small and possibly biased sample, our analysis and conclusions necessarily remain tentative.

Analysis and Life History Parameters

The Models

The availability of data limits the number of parameters that can rationally be used in a model. Our initial efforts utilized an aggregated age-group model having three age-classes: calves, immatures, and mature animals (Braham and Breiwick 1980). In using this model, it was necessary to postulate a "fraction maturing" annually, which is associated with the age at maturity. This approach was attempted because age determination is not currently possible for bowheads (Nerini et al. 1984), and thus the catch could be represented only by two classes. However, the fraction maturing depends on current age structure, so we have here reverted to the usual age structured Leslie model (Lewis 1942; Leslie 1945, 1948).

Different versions of the basic model were used in an effort to limit the number of parameters that have to be assumed. Since details of age structure are unknown, we first assumed a stable age structure and then checked the outcomes by calculations that utilized the recorded harvests. In the initial version, the Leslie matrix was "collapsed" to a single entry for mature (reproducing) animals. With this version, the available life history data (calf counts and estimated proportion of immature bowheads) suggested very high adult survival rates, so that an extended version (full matrix) was used with an upper age limit assumed to approximate senescence.

In the first model, five parameters were used: (1) an annual immature survival rate (s_0) prevailing from age 1 to age a, (2) a mature (adult) survival rate (s) applied annually from age $a + 1$ onwards, (3) the age of sexual maturity (m), (4) a fecundity value (f), and (5) the age of transition to adult mortality (a). Under the arrangement used here, f is the product of birth rate and 1st yr survival. Parturition is assumed to occur 1 yr after an individual reaches the age of sexual maturity. The age vector thus contains $m + 1$ elements, ranging from $n_{1,t}$ (1-yr-olds) to the age of sexual maturity ($n_{m,t}$), plus a final class ($n_{m+1,t}$) containing all of the fully mature individuals. The usual application of a Leslie matrix considers only females. Since we assume a 1:1 sex ratio in the catch and in the population (discussed below), we use total catch and total population size, and the factor of $\frac{1}{2}$ necessary to reduce total estimates to females can be cancelled out of the relevant equations. The projection matrix then is

$$A_1 = \begin{array}{c} \left[\begin{array}{cccccccc} 0 & 0 & \cdots & & & 0 & 0 & f \\ s_0 & 0 & \cdots & & & 0 & 0 & 0 \\ 0 & s_0 & & & & & & \\ & & \ddots & & & & & \\ & & & s_0 & & & & \\ & & & & s & & & \\ & & & & & \ddots & & \\ 0 & 0 & \cdots & & & s & 0 & 0 \\ 0 & 0 & \cdots & & & 0 & s & s \end{array}\right] \\ \begin{array}{cccccccc} 1 & 2 & \cdots & a & a+1 & \cdots & m & m+1 \end{array} \end{array}$$

where $f = s_0'F$, s_0' = calf survival rate, and F = number of female calves produced per mature female (= number of calves produced per mature animal).

The characteristic equation of the above matrix A_1 is

(1) $\lambda^{m+1} - s\lambda^m - s_0{}^a s^{m-a} f = 0$

where λ is the eigenvalue or "population multiplier," $N_{t+1} = \lambda N_t$. The characteristic equation assumes a stable age distribution and subsumes the effect of catches in the survival rates, s_0 and s.

The extended version has the following matrix:

$$A_2 = \begin{array}{c} \left[\begin{array}{cccccccccc} 0 & 0 & \cdots & & 0 & 0 & f & \cdots & f & 0 \\ s_0 & 0 & \cdots & & 0 & 0 & 0 & \cdots & 0 & 0 \\ 0 & s_0 & & & & & & & & \\ & & \ddots & & & & & & & \\ & & & s_0 & & & & & & \\ & & & & s & & & & & \\ & & & & & s & & & & \\ & & & & & & \ddots & & & \\ & & & & & & & s & & \\ & & & & & & & & \ddots & \\ 0 & 0 & \cdots & & & & & & s & 0 \end{array}\right] \\ \begin{array}{cccccccccc} 1 & 2 & \cdots & a & \cdots & m & m+1 & \cdots & w-1 & w \end{array} \end{array}.$$

Parturition occurs at age $m + 1$, as before, and extends to age $w - 1$. All individuals aged w at time t die before time $t + 1$. The other parameters are as described above. The characteristic equation of matrix A_2 is

(2) $\lambda^{m+1} - s\lambda^m - s_0{}^a s^{m-a} f[1 - (s/\lambda)^{w-m-1}] = 0.$

To use the matrices for projections, s_0 and s are redefined as representing natural mortality only. Catch data since 1961 indicated that approximately 95% of the annual harvest occurred within a 4-wk period each spring. We therefore assume that no natural mortality occurs during the period of the harvest and remove catches annually by simply reducing the appropriate age vector by the catch (at age). Catch is thus incorporated into the model in the following manner:

(3) $N_{t+1} = A_2(N_t - H_t)$

where the vector of catches is

$$H_t = [c_{1,t}, c_{2,t}, \ldots, c_{w-1,t}, c_{w,t}].$$

A computer program (using APL and FORTRAN) was written to calculate results from the various systems of equations for given input parameters. We did not examine the effect of secular trends in the parameters and used constant values for each year. The effect of uncertainty about actual values of the parameters was examined by assuming ranges of values and comparing the resulting projections. Current information about the needed parameters is summarized in the following sections.

Density Dependence

The matrix (A_2) was used to project the population from 1848, when commercial whaling began, to 1970. Presumably the preexploitation (1848) population was in equilibrium, with annual birth and death rates approximately equal. It would then be expected that the much smaller population at the end of commercial exploitation (about 1914) would have a positive rate of growth, owing to density compensation, at least in the absence of further exploitation. A density-dependent relationship was adopted based on the modified logistic growth curve currently employed by the IWC. The usual assumption (Allen 1980, p. 40) is

(4) $r_t = M + (r - M)_{\max}[1 - (P_t/P_0)^z]$

where r_t denotes net recruitment rate in year t, M represents natural mortality, P_t is current population size, P_0 is the unexploited population size, z is an exponent equal to or greater than unity, and $(r - M)_{\max}$ is the maximum net recruitment rate.

We have assumed that the above equation operates on recruitment to age 1 (f) in the Leslie matrix. Since this rate (f) is the product of calving rate (F) and survival to age 1 (s_0'), the density dependency can be considered to be mediated either through changes in reproductive rate or 1st yr survival; we have no evidence as to the nature of the actual mechanism. The assumption of a stationary (constant) population in 1848 implies that recruitment (f) in that year may be calculated from equation (2) with $\lambda = 1$. It seems quite reasonable to suppose that a stable age distribution would have existed then and to neglect the small proportion of the population taken in the Eskimo hunts (so that prevailing survival rates were essentially determined by natural causes). Recruitment in any future year was then computed from

(5) $f_t = f_0 + (f_{\max} - f_0)[1 - (P_t/P_0)^z]$

where f_0 is the recruitment rate in 1848:

(6) $f_0 = (1 - s)/s_0{}^a s^{m-a}[1 - s^{w-m-1}].$

We have thus introduced two further parameters, z and $f_{\max}$. In any given computation of a series of populations from 1848 to 1970, the parameter actually appearing in equations is maximum net recruitment (productivity) rate, so we denote this as

(7) $r_m = (f_{\max} - f_0) = f_{\max} - [(1 - s)/(s_0/s)^a(s^m - s^{w-1})].$

In the calculations, we used a range of maximum net productivity of 0.01–0.05, i.e. $0.01 \leqslant r_m \leqslant 0.05$.

Sex Ratio

The male to female sex ratio of bowheads taken at Barrow during 1961–73 was 1:1 (Durham 1979). More males than females have been landed in the Eskimo harvest since 1973 although the 1973–82 pooled sex ratio (0.83:1.00) does not depart significantly from unity ($\chi^2 = 1.94$, $P = 0.16$) (Nerini et al. 1984). We conclude that the sex ratio of the harvested population is 1:1 and assume that this is representative of the entire population. We also assume an even sex ratio for struck and lost animals based on a sample of 12 animals struck and lost and subsequently found dead as "stinkers" (NMFS, unpubl. data).

Length at Sexual Maturity

Female bowhead whales become sexually mature at approximately 13–14 m in length (Nerini et al. 1984). Davis et al. (1983) noted that the smallest presumed female associated with a calf was 12.2 m and that 91% of females with calves were greater than 13.5 m. Both ovaries from 18 females whose body lengths ranged from 6.9 to 16.2 m, collected from 1976 to 1980, were examined for the presence of corpora albicantia (Nerini et al. 1984). All females greater than 13.5 m (seven) had previously ovulated. In the absence of an adequate aging method, length at maturity for the female component of the population can be used to reconstruct the reproductive status of the population.

Length–frequency data for the population are also needed. During aerial surveys of the spring migrations (April and May) of 1979 and 1980, 127 whales (noncalves) were scored as to relative size. These data suggest that about 60% of the population older than calves were greater than 12 m (Johnson et al. 1981). A study by the Naval Oceans Systems Center (D. Ljungblad, Naval Ocean Systems Center, Marine Sciences Division, San Diego, CA 92152, pers. comm.) also indicated that the fraction mature was on the order of 60%. Davis et al. (1983) sampled the summer population in the Canadian Beaufort Sea in 1982 and found that 42% (162 of 390) of the animals older than calves were mature (>13 m). They pointed out, however, that this sampling may have been biased because of possible segregation of various age- and sex-classes.

Proportion of Calves

There has been controversy surrounding the estimation of gross annual recruitment rate for bowheads. Braham et al. (1979) reported that the minimum fraction of observed calves was 0.025–0.035. Davis and Koski (1980) presented similar results for bowhead whales in the eastern Canadian Arctic. This range of estimates came from data collected only during the spring migration (April–June) and did not account for calves possibly born after early June. Evidence presented in Marquette (1977), Nerini et al. (1984), Davis et al. (1983), and anecdotal Eskimo accounts suggested that calving may extend into August. It is also likely that some calves were not seen in the spring migration. Several independent estimates of the proportion of calves in the population made from 1976 to 1982 by various investigators are shown in Nerini et al. (1984). These estimates range from 0.010 to 0.124, with most in the range 0.025–0.035. However, most of the estimates were based on small sizes and some were based on sampling before the end of the calving season. Davis et al. (1983), using photogrammetry in the Canadian Beaufort Sea during August, obtained an estimate of 0.093–0.124 as the fraction of calves, and Ljungblad et al. (1983) reported a fraction of 0.058 for July–October 1982 in the United States Beaufort and Bering seas. Nerini et al. (1984), incorporating the new findings from 1982, proposed that the proportion of calves lies in the range 0.036–0.116.

As noted above, the fecundity value for the Leslie matrix used here consists of the product of the birth rate and 1st yr survival, inasmuch as the first class of the age vector consists of 1-yr-old whales. If the minimum fraction of calves proposed by Nerini et al. (1984) is adjusted for proportion of adults in the population ($0.036/0.60 = 0.060$), and assuming 1st yr survival to be 0.85, then $f = 0.05$ is a prospective minimum value. For an upper limit, we consider a 3-yr birth interval and an even sex ratio, giving a birth rate of $(0.333)(0.5) = 0.17$. This estimate needs to be reduced to allow for 1st yr survival, so that maintaining it as an upper limit allows for the possibility of a somewhat shorter birth interval. Right whales (*Balaena glacialis*) off Argentina appear to have a 3-yr mean calving interval, with a range of 2–7 yr, and an apparent gross annual reproductive rate of 6–7% (R. Payne, Center for Long Term Research, Inc., Weston Road, Lincoln, MA 01773, pers. comm.). We thus assume that f lies in the range of 0.05–0.17. Since recent populations are much reduced from preexploitation levels, the assumption of equation (5) means that current levels of productivity (f_t) will be quite close to maximal values ($f_{\max}$). We have used values of $f_{\max}$ of 0.07, 0.10, and 0.13 in the calculations.

Age at Sexual Maturity

The age of sexual maturity is not known for bowheads. A range of values of 5, 7, and 9 yr, selected to correspond to other modeling studies, was considered. Breiwick et al. (1980), in their analysis of possible initial population sizes of this stock, found the model to be relatively insensitive to changes in the lag time between birth and sexual maturity. The same conclusion about ages at sexual maturity results from the present study.

Survival

Natural mortality has not yet been directly estimated for bowheads. Ohsumi (1979; S. Ohsumi, Far Seas Fisheries Research Laboratory, 1000 Orido, Shimizu, Japan, pers. comm.) provided an indirect estimate of natural mortality from correlations among baleen whales, using average body length of mature animals. From his length–mortality curve we assume that a realistic range of adult bowhead instantaneous natural mortality rates (M) is 0.03–0.07 based on an average mature body length of 15.2 m. Breiwick et al. (1980) used a range of 0.04–0.08 based on estimates for other species of baleen whales. Immature natural mortality rates also are not known. Allen (1980, p. 10) suggested that there may be little difference between adult and immature rates (note that 1st yr survival is not involved here). We have assumed that adult natural mortality rates are such that annual survival rates are in the range 0.92–0.98. We have constrained immature survival rates not to exceed those of adults, and we also use the restriction that immature survival rates do not differ from those of adults by more than 5%, i.e. that $(s - s_0) \leq 0.05$. The age (a) at which immature survival approaches that of adults is unknown, so we have adopted values of 1 and 3 yr. Thus, three survival rates are assumed: a rate from birth to age 1 (implicit in the parameter f), an immature rate (s_0) to age a, and an adult rate (s). The age of senescence is not known for bowheads, so reasonable-seeming values of $w = 50$ and $w = 75$ were adopted.

Catch composition

Of 119 whales landed between 1974 and 1981, 79% were 14 m or less in length. Those below 14 m were considered the immature group (Nerini et al. 1984). Durham (1979) reported a frequency of 81% for immatures from his 1961–73 data. Hence,

TABLE 1. Pelagic and shore-based kill and struck and lost (S/L) data for the western Arctic bowhead whale (see table footnotes for data sources and text for explanation of struck and lost rates used).

Season	Pelagic kill	Pelagic S/L	Pelagic total	Alaskan and Canadian shore kill	Soviet shore kill	Shore S/L	Total shore kill	Total kill	Cumulative total kill
1848[a]	15	3	18	0	0	0	0	18	18
1849[b]	507	64	571	0	1	1	2	573	591
1850	1719	348	2067	0	0	0	0	2067	2 658
1851	757	139	896	0	1	1	2	898	3 556
1852	2188	494	2682	17	1	9	27	2709	6 265
1853	628	168	796	7	0	4	11	807	7 072
1854	105	25	130	24	0	12	36	166	7 238
1855	0	2	2	0	0	0	0	2	7 240
1856	0	0	0	0	0	0	0	0	7 240
1857	72	6	78	0	0	0	0	78	7 318
1858	424	35	459	0	0	0	0	459	7 777
1859	335	31	366	0	4	2	6	372	8 149
1860	211	10	221	0	0	0	0	221	8 370
1861	293	13	306	0	0	0	0	306	8 676
1862	150	7	157	0	0	0	0	157	8 833
1863	288	15	303	0	0	0	0	303	9 136
1864	396	38	434	0	0	0	0	434	9 570
1865	455	133	588	0	1	1	2	590	10 160
1866	503	37	540	0	9	5	14	554	10 714
1867	566	33	599	0	0	0	0	599	11 313
1868	456	60	516	0	0	0	0	516	11 829
1869	340	30	370	6	2	4	12	382	12 211
1870	594	26	620	5	6	6	17	637	12 848
1871	125	8	133	0	3	2	5	138	12 986
1872	163	31	194	4	0	2	6	200	13 186
1873	147	0	147	0	0	0	0	147	13 333
1874	95	0	95	0	0	0	0	95	13 428
1875	200	0	200	0	0	0	0	200	13 628
1876	57	19	76	0	0	0	0	76	13 704
1877	244	18	262	0	5	3	8	270	13 974
1878	72	8	80	0	0	0	0	80	14 054
1879	203	58	261	3	0	2	5	266	14 320
1880	452	8	460	12	1	7	20	480	14 800
1881	374	44	418	11	0	6	17	435	15 235
1882	240	0	240	1	0	1	2	242	15 477
1883	39	0	39	2	0	1	3	42	15 519
1884	114	19	133	10	5	8	23	156	15 675
1885	277	10	287	41	19	30	90	377	16 052
1886	123	10	133	8	10	9	27	160	16 212
1887	180	24	204	23	1	12	36	240	16 452
1888	117	16	133	18	0	9	27	160	16 612
1889	42	11	53	49	0	25	74	127	16 739
1890	127	0	127	6	0	3	9	136	16 875
1891	228	6	234	32	0	16	48	282	17 157
1892	308	9	317	17	0	9	26	343	17 500
1893	141	0	141	26	0	13	39	180	17 680
1894	141	10	151	48	3	26	77	228	17 908
1895	94	0	94	10	5	8	23	117	18 025
1896	58	0	58	40	0	20	60	118	18 143
1897	73	0	73	38	0	19	57	130	18 273
1898	216	12	228	41	9	25	75	303	18 576
1899	204	4	208	10	3	7	20	228	18 804
1900	112	0	112	20	3	12	35	147	18 951
1901	29	0	29	14	0	7	21	50	19 001
1902	132	0	132	18	2	10	30	162	19 163
1903	95	0	95	8	1	5	14	109	19 272
1904	68	6	74	5	0	3	8	82	19 354
1905	86	7	93	8	0	4	12	105	19 459
1906	36	0	36	17	3	10	30	66	19 525
1907	70	0	70	9	2	6	17	87	19 612
1908	33	0	33	48	11	30	89	122	19 734
1909	10	0	10	25	6	16	47	57	19 791

TABLE 1. (*Continued*)

Season	Pelagic kill	Pelagic S/L	Pelagic total	Alaskan and Canadian shore kill	Soviet shore kill	Shore S/L	Total shore kill	Total kill	Cumulative total kill
1910	16	0	16	9	0	5	14	30	19 821
1911	30	0	30	7	0	4	11	41	19 862
1912	0	0	0	18	0	9	27	27	19 889
1913	0	0	0	5	0	3	8	8	19 897
1914	40	0	40	9	0	5	14	54	19 951
1915	0	0	0	4	0	2	6	6	19 957
1916	0	0	0	16	0	8	24	24	19 981
1917	0	0	0	15	0	8	23	23	20 004
1918	0	0	0	18	0	9	27	27	20 031
1919	16	0	16	7	0	4	11	27	20 058
1920	0	0	0	16	5	11	32	32	20 090
1921	0	0	0	5	0	3	8	8	20 098
1922	0	0	0	26	0	13	39	39	20 137
1923	0	0	0	5	1	3	9	9	20 146
1924	0	0	0	24	2	13	39	39	20 185
1925	0	0	0	32	2	17	51	51	20 236
1926	0	0	0	20	2	11	33	33	20 269
1927	0	0	0	8	2	5	15	15	20 284
1928	0	0	0	15	4	10	29	29	20 313
1929	0	0	0	17	3	10	30	30	20 343
1930	0	0	0	8	3	6	17	17	20 360
1931	0	0	0	18	2	10	30	30	20 390
1932	0	0	0	7	9	8	24	24	20 414
1933	0	0	0	5	9	7	21	21	20 435
1934	0	0	0	4	8	6	18	18	20 453
1935	0	0	0	7	2	5	14	14	20 467
1936	0	0	0	10	5	8	23	23	20 490
1937	0	0	0	16	3	10	29	29	20 519
1938	0	0	0	13	2	8	23	23	20 542
1939	0	0	0	8	0	4	12	12	20 554
1941	0	0	0	23	2	13	38	38	20 610
1942	0	0	0	11	3	7	21	21	20 631
1943	0	0	0	7	2	5	14	14	20 645
1944	0	0	0	2	0	1	3	3	20 648
1945	0	0	0	12	3	8	23	23	20 671
1946	0	0	0	12	1	7	20	20	20 691
1947	0	0	0	11	0	6	17	17	20 708
1948	0	0	0	5	0	3	8	8	20 716
1949	0	0	0	6	0	3	9	9	20 725
1950	0	0	0	9	0	5	14	14	20 739
1951	0	0	0	14	0	7	21	21	20 760
1952	0	0	0	8	0	4	12	12	20 772
1953	0	0	0	23	0	12	35	35	20 807
1954	0	0	0	5	0	3	8	8	20 815
1955	0	0	0	23	0	12	35	35	20 850
1956	0	0	0	7	0	4	11	11	20 861
1957	0	0	0	3	0	2	5	5	20 866
1958	0	0	0	2	1	2	5	5	20 871
1959	0	0	0	1	0	1	2	2	20 873
1960	0	0	0	19	0	10	29	29	20 902
1961	0	0	0	11	0	6	17	17	20 919
1962	0	0	0	13	0	7	20	20	20 939
1963	0	0	0	10	0	5	15	15	20 954
1964	0	0	0	16	0	8	24	24	20 978
1965	0	0	0	9	0	5	14	14	20 992
1966	0	0	0	15	0	8	23	23	21 015
1967	0	0	0	7	0	4	11	11	21 026
1968	0	0	0	17	0	9	26	26	21 052
1969	0	0	0	21	0	11	32	32	21 084
1970[c]	0	0	0	29	0	37	48	48	21 132
1971	0	0	0	24	0	3	26	26	21 158
1972	0	0	0	40	1	3	43	43	21 201
1973	0	0	0	38	2	15	48	48	21 249

TABLE 1. (*Concluded*)

Season	Pelagic kill	Pelagic S/L	Pelagic total	Alaskan and Canadian shore kill	Soviet shore kill	Shore S/L	Total shore kill	Total kill	Cumulative total kill
1974	0	0	0	23	3	28	40	40	21 289
1975[d]	0	0	0	17	2	26	32	32	21 321
1976[e]	0	0	0	56	0	35	74	74	21 395
1977	0	0	0	32	0	79	72	72	21 467
1978[f]	0	0	0	13	0	5	17	17	21 484
1979[g]	0	0	0	12	0	15	23	23	21 507
1980[h]	0	0	0	19	0	15	30	30	21 537
1981[j]	0	0	0	19	0	9	26	26	21 563
1982[j]	0	0	0	8	0	11	16	16	21 579

[a] Pelagic kill and struck and lost data for 1848 was taken from Breiwick and Mitchell (1983). Pelagic data from Bockstoce and Botkin (1983) were used for the period 1849–1914. The 1919 pelagic kill of 16 is a personal communication to G. H. Jarrell from T. B. Brower Sr., 4 January 1983.

[b] Shore kill from Marquette and Bockstoce (1980, tables 2, 3, and 5), supplemented by Bogoslovskaya et al. (1982, table 2). There is much duplication in the two sources for Soviet shore kill between 1920 and 1938. If a value was given for a range of years, an average value was assigned to each year. Fractional values were added and rounded to the nearest integer.

[c] 1970–77 data from Marquette and Bockstoce (1980).

[d] Includes 3 struck and lost accidentally omitted from Marquette and Bockstoce (1980) (see Marquette 1976).

[e] A typographical error in Marquette and Bockstoce (1980) resulted in a struck and lost of 38 (see Marquette 1978).

[f] Spring data from Braham et al. (1979); fall data from Braham et al. (1980b).

[g] Spring data from Braham et al. (1980a); fall data from Johnson et al. (1981).

[h] Spring data from Johnson et al. (1981); fall data from Marquette et al. (1982).

[i] Spring data from Marquette et al. (1982); fall data from Dronenburg et al. (1983).

[j] Spring data from Dronenburg et al. (1983); fall data from Dronenburg et al. (1984).

we use an estimate of 80% of the harvest being taken from immature age-classes for the period from the cessation of commercial whaling (1914) to the present. However, the commercial catch may have been biased towards larger whales, so, for lack of better estimates, we have assumed a reversal of the fractions for the period 1848–1914, i.e. 80% of the catch came from mature age-classes in that period. In each case we have prorated the catch to the age-classes according to the relative abundance of that class at the start of the season.

Mortality of Struck and Lost Whales

Whales have been known to recover from a strike even when a bomb has gone off inside an animal (Cook 1926; Albert et al. 1980; Johnson et al. 1981). However, an unknown fraction of the whales struck but lost eventually die (Marquette 1977; Braham et al. 1979; Braham et al. 1980a; Marquette et al. 1982). The rate of recovery of stinkers has been about 5–7% since 1974 (NMFS, unpubl. data). Durham (1979) estimated recovered stinkers at 10–20% of the catch, but this only provides a minimum estimate of mortality and does not include those stinkers not recovered.

We have used the total kill data shown in Table 1. For the shore kill from 1848 to 1969, we used a 50% struck and lost rate (i.e. the number of animals struck and lost equals 50% of the known or estimated kill), based on limited data from Mitchell (1977), and a corresponding 100% mortality rate for whales struck and lost. The assumption of a 100% mortality rate for struck and lost whales for this period may seem unrealistic but much of the shore-based catch statistics are still incomplete so that the two effects may compensate for each other (IWC 1982). For the period 1970–77 we used the actual number of whales struck and lost and have assumed that a 50% mortality rate resulted from striking and losing a whale. However, given that the hunt today is under more rigorous control and that greater effort is being given to making strikes more effective, mortality of struck and most animals is probably greater now than during 1970–77. The estimated mortality rate of struck and lost animals, based on limited data, was 80% in spring 1981 (Marquette et al. 1982) and 50% in spring 1982 (Dronenburg et al. 1983). From 1978 to 1982 we assumed a 75% struck and lost mortality rate.

Results and Discussion

Initial Explorations

We conducted rather extensive investigations into the outcomes of assuming various 1914 populations projected forward to 1970, as done by Eberhardt and Breiwick (1980). Many of these initial calculations utilized the simple collapsed matrix A_1, but the main calculations are now based on the "full" matrix A_2. The general approach was partly inspired by the need to utilize any available information about the population. One such item is the observed proportion of the population less than the estimated length at sexual maturity. As discussed in the section above on length at sexual maturity, it was estimated that about 40% of the population was sexually immature.

If a stable age distribution is assumed, an equation for the proportion of immatures can be set up using the parameters of the matrix A_2, other than f, which is not involved. Terms of the stable age distribution from age 1 through age of sexual maturity are proportional to

$$1,\ s_0/\lambda,\ (s_0/\lambda)^2,\ \ldots,\ (s_0/\lambda)^a,\ (s_0/\lambda)^a(s/\lambda),\ \ldots,\ (s_0/\lambda)^a(s/\lambda)^{m-a-1}$$

and these sum to

$$I = [(1-(s_0/\lambda)^{a+1})/(1-s_0/\lambda)] + (s_0/\lambda)^a(s/\lambda)[1-(s/\lambda)^{m-a-1}]/(1-s/\lambda).$$

TABLE 2. Projection results for the combination $a = 3$, $m = 9$, $w = 50$ (Im_0 and Im_1 are fraction of immatures in 1848 and 1970, respectively, P_{1848} = 1848 population size, $P_{\min}$ = minimum population size from 1848 to 1970, $Y_{\min}$ = the year in which $P_{\min}$ occurred, $P_n = 4000/P_{1848}$, P_{c0}, P_{c20}, and P_{c40} are the year 2000 population sizes for an annual kill of 0, 20, and 40 bowheads from 1983 to 2000).

$f_{\max}$	r_m	s_0	s	Im_0	Im_1	P_{1848}	$P_{\min}$	$Y_{\min}$	P_n	P_{c0}	P_{c20}	P_{c40}
0.07	0.0104	0.95	0.97	0.310	0.332	20 875	3752	1915	0.19	4211	3873	3537
	0.0122	0.96	0.97	0.308	0.336	20 246	3531	1915	0.20	4382	4040	3699
	0.0140	0.97	0.97	0.307	0.340	19 669	3318	1912	0.20	4559	4213	3868
	0.0194	0.93	0.98	0.269	0.318	18 311	2813	1910	0.22	5055	4698	4343
	0.0210	0.94	0.98	0.267	0.322	17 879	2644	1910	0.22	5260	4899	4539
	0.0225	0.95	0.98	0.266	0.326	17 478	2487	1910	0.23	5473	5107	4743
	0.0240	0.96	0.98	0.264	0.330	17 105	2342	1910	0.23	5693	5322	4953
	0.0254	0.97	0.98	0.263	0.334	16 756	2207	1910	0.24	5920	5545	5171
	0.0267	0.98	0.98	0.261	0.338	16 430	2083	1910	0.24	6154	5775	5397
0.10	0.0157	0.91	0.96	0.363	0.391	20 775	3551	1915	0.19	4368	4039	3712
	0.0184	0.92	0.96	0.361	0.395	20 106	3320	1912	0.20	4561	4228	3896
	0.0210	0.93	0.96	0.359	0.399	19 496	3101	1912	0.21	4763	4425	4088
	0.0235	0.94	0.96	0.357	0.403	18 938	2899	1912	0.21	4972	4629	4287
	0.0259	0.95	0.96	0.355	0.408	18 426	2713	1912	0.22	5189	4842	4495
	0.0282	0.96	0.96	0.353	0.412	17 954	2541	1910	0.22	5414	5062	4711
	0.0343	0.92	0.97	0.315	0.389	16 966	2200	1910	0.24	5925	5564	5205
	0.0364	0.93	0.97	0.313	0.393	16 592	2064	1910	0.24	6182	5817	5453
	0.0384	0.94	0.97	0.312	0.397	16 244	1938	1910	0.25	6449	6079	5710
	0.0404	0.95	0.97	0.310	0.401	15 918	1823	1910	0.25	6725	6351	5977
	0.0422	0.96	0.97	0.308	0.405	15 612	1716	1910	0.26	7010	6631	6253
	0.0440	0.97	0.97	0.307	0.410	15 325	1618	1910	0.26	7304	6921	6538
	0.0494	0.93	0.98	0.269	0.387	14 742	1421	1910	0.27	7967	7579	7191
0.13	0.0109	0.93	0.94	0.449	0.465	23 007	3960	1943	0.17	4049	3732	3416
	0.0157	0.94	0.94	0.447	0.469	22 119	3743	1915	0.18	4240	3918	3597
	0.0229	0.90	0.95	0.411	0.446	20 535	3293	1915	0.19	4592	4264	3938
	0.0264	0.91	0.95	0.409	0.450	19 864	3063	1912	0.20	4809	4476	4145
	0.0298	0.92	0.95	0.407	0.454	19 253	2851	1912	0.21	5034	4697	4360
	0.0330	0.93	0.95	0.405	0.458	18 695	2656	1912	0.21	5269	4927	4585
	0.0360	0.94	0.95	0.403	0.462	18 184	2478	1912	0.22	5514	5166	4819
	0.0390	0.95	0.95	0.401	0.467	17 713	2314	1912	0.23	5769	5416	5063
	0.0457	0.91	0.96	0.363	0.444	16 822	2041	1910	0.24	6242	5883	5525
	0.0484	0.92	0.96	0.361	0.448	16 440	1909	1910	0.24	6530	6166	5802

The terms for mature animals are

$$(s_0/\lambda)^a(s/\lambda)^{m-a}[1,\ s/\lambda,\ (s/\lambda)^2,\ \ldots,\ (s/\lambda)^{w-m-1}]$$

summing to

$$A = (s_0/\lambda)^a(s/\lambda)^{m-a}[1 - (s/\lambda)^{w-m}]/(1 - s/\lambda).$$

Hence, the proportion of immatures in a stable population is given by $I/(I + A)$. We calculated λ from the assumed 1914 populations and then used the equation for proportion of immatures and the characteristic equation (equation (2)) to eliminate one of the unknown parameters (s or s_0). In effect, one of these parameters is replaced by the proportion of immatures. These calculations were helpful in assessing the likely validity of the observed proportion of immatures, which appears to be too high for observed values of the other parameters. After extensive explorations (Breiwick et al. 1982), it became evident that a better procedure might be to use the catch history to project from 1848 to 1970 and then to reassess the likely course of events from the low points of those trajectories to recent times.

Due to our lack of knowledge of any secular trend in the parameters, we have used constant values throughout time in any given run of the model. We have ignored unknown factors such as environmental variability (cf. Beddington and Grenfell 1979; Nisbet and Gurney 1982) and year-to-year variability in the parameters. However, we recognize that such variability may be important. For example, a mass mortality of "several scores" of bowheads occurred in Karaginskii Bay, USSR, in 1982 (Sleptsov 1948, cited in Tomilin 1957 (in text but no citation given, see Mitchell and Reeves 1982, p. 78, for citation)). This type of mortality could be of major importance (Mitchell and Reeves 1982).

Projections from 1848 to 1970

These projections assume a 1970 population of 4000 whales and the parameter ranges given in discussions of life history parameters in the sections above. We use 4000 whales in 1970 rather than the approximately 3800 of recent estimates both to be conservative with respect to the higher harvest in years subsequent to 1970 and because current populations likely are larger than estimated. An inspection of equation (7) shows that maximum net recruitment will be maximized for small values of a and m, combined with large values of w. Conversely, large values of a and m with small values of w minimize r_m. Consequently, the major outcomes of the projections can be obtained by inspecting the results of parameter sets $a = 1$, $m =$

TABLE 3. Projection results for the combination $a = 1$, $m = 5$, $w = 75$ (Im_0 and Im_1 are fraction of immatures in 1848 and 1970, respectively, P_{1848} = 1848 population size, $P_{\min}$ = minimum population size from 1848 to 1970, $Y_{\min}$ = the year in which $P_{\min}$ occurred, $P_n = 4000/P_{1848}$, P_{c0}, P_{c20}, and P_{c40} are the year 2000 population sizes for an annual kill of 0, 20, and 40 bowheads from 1983 to 2000).

$f_{\max}$	r_m	s_0	s	Im_0	Im_1	P_{1848}	$P_{\min}$	$\mathrm{Y}_{\min}$	P_n	P_{c0}	P_{c20}	P_{c40}
0.07	0.0150	0.91	0.96	0.196	0.226	19 089	3144	1912	0.21	4763	4422	4082
	0.0156	0.92	0.96	0.195	0.227	18 889	3074	1912	0.21	4833	4490	4148
	0.0161	0.93	0.96	0.195	0.229	18 697	3005	1912	0.21	4903	4559	4215
	0.0167	0.94	0.96	0.194	0.230	18 511	2938	1912	0.22	4974	4628	4283
	0.0173	0.95	0.96	0.194	0.231	18 331	2874	1912	0.22	5046	4698	4351
	0.0178	0.96	0.96	0.194	0.232	18 157	2812	1912	0.22	5118	4769	4420
	0.0280	0.92	0.97	0.159	0.224	15 910	1976	1910	0.25	6429	6057	5684
	0.0285	0.93	0.97	0.158	0.226	15 794	1935	1910	0.25	6521	6147	5773
	0.0289	0.94	0.97	0.158	0.227	15 682	1896	1910	0.26	6613	6237	5862
	0.0294	0.95	0.97	0.158	0.228	15 572	1857	1910	0.26	6706	6329	5951
	0.0298	0.96	0.97	0.158	0.229	15 466	1820	1910	0.26	6800	6421	6042
	0.0302	0.97	0.97	0.157	0.231	15 362	1784	1910	0.26	6894	6513	6133
	0.0390	0.93	0.98	0.124	0.223	14 018	1302	1910	0.29	8555	8162	7766
	0.0393	0.94	0.98	0.124	0.224	13 941	1279	1910	0.29	8666	8273	7877
	0.0396	0.95	0.98	0.124	0.225	13 866	1256	1910	0.29	8777	8384	7987
	0.0400	0.96	0.98	0.124	0.226	13 792	1234	1910	0.29	8887	8494	8098
	0.0403	0.97	0.98	0.123	0.228	13 720	1213	1910	0.29	8996	8604	8208
	0.0406	0.98	0.98	0.123	0.229	13 649	1192	1910	0.29	9105	8714	8318
0.10	0.0124	0.89	0.94	0.271	0.290	20 776	3643	1915	0.19	4346	4023	3700
	0.0134	0.90	0.94	0.271	0.292	20 474	3546	1915	0.20	4423	4098	3773
	0.0144	0.91	0.94	0.270	0.293	20 185	3452	1915	0.20	4501	4174	3847
	0.0153	0.92	0.94	0.270	0.294	19 908	3361	1915	0.20	4580	4250	3922
	0.0162	0.93	0.94	0.269	0.296	19 643	3274	1915	0.20	4659	4328	3998
	0.0171	0.94	0.94	0.269	0.297	19 389	3189	1912	0.21	4740	4407	4074
	0.0298	0.90	0.95	0.233	0.288	16 720	2262	1910	0.24	5904	5549	5195
	0.0305	0.91	0.95	0.233	0.290	16 556	2204	1910	0.24	6006	5650	5293
	0.0313	0.92	0.95	0.232	0.291	16 398	2148	1910	0.24	6109	5751	5393
	0.0320	0.93	0.95	0.232	0.293	16 245	2094	1910	0.25	6214	5853	5493
	0.0327	0.94	0.95	0.232	0.294	16 097	2042	1910	0.25	6319	5956	5594
	0.0334	0.95	0.95	0.231	0.295	15 954	1993	1910	0.25	6425	6061	5697
	0.0450	0.91	0.96	0.196	0.286	14 370	1458	1910	0.28	7933	7554	7173
	0.0456	0.92	0.96	0.195	0.288	14 267	1425	1910	0.28	8060	7680	7298
	0.0461	0.93	0.96	0.195	0.289	14 166	1394	1910	0.28	8188	7807	7425
	0.0467	0.94	0.96	0.194	0.291	14 068	1363	1910	0.28	8316	7935	7551
	0.0473	0.95	0.96	0.194	0.292	13 973	1334	1910	0.29	8444	8063	7679
	0.0478	0.96	0.96	0.194	0.293	13 880	1305	1910	0.29	8571	8190	7806
0.13	0.0229	0.88	0.93	0.308	0.341	19 020	2981	1912	0.21	4952	4620	4289
	0.0242	0.89	0.93	0.308	0.342	18 753	2889	1912	0.21	5051	4718	4384
	0.0253	0.90	0.93	0.307	0.344	18 498	2801	1912	0.22	5153	4816	4481
	0.0265	0.91	0.93	0.307	0.345	18 254	2717	1912	0.22	5255	4917	4579
	0.0276	0.92	0.93	0.306	0.347	18 020	2637	1912	0.22	5359	5018	4678
	0.0287	0.93	0.93	0.306	0.348	17 796	2559	1912	0.22	5464	5121	4779
	0.0424	0.89	0.94	0.271	0.339	15 726	1865	1910	0.25	6727	6365	6002
	0.0434	0.90	0.94	0.271	0.340	15 572	1813	1910	0.26	6858	6494	6129
	0.0444	0.91	0.94	0.270	0.342	15 424	1763	1910	0.26	6990	6624	6258
	0.0453	0.92	0.94	0.270	0.343	15 280	1715	1910	0.26	7123	6756	6387
	0.0462	0.93	0.94	0.269	0.345	15 141	1669	1910	0.26	7258	6889	6518
	0.0471	0.94	0.94	0.269	0.346	15 007	1625	1910	0.27	7394	7023	6651

5, $w = 75$ and $a = 3$, $m = 9$, $w = 50$. The actual projections employed equation (5) and the Leslie matrix A_2 in an iterative procedure, so that the initial population in 1848 is varied until a 1970 population of 4000 is achieved, with removals following the catch history (Table 1, column 9). Two values of z were studied, one ($z = 1$) giving the ordinary logistic growth curve, while the second ($z = 5.04$) yielded a maximum net productivity point of about 70%, a little above that frequently employed in IWC calculations (Allen 1980). Our main results are based on the assumption of the larger exponent. Using lower values of z tends to increase the 1848 population sizes as well as the minimum level at the cessation of pelagic whaling.

Estimates obtained from the projections (Table 2 and 3) included the initial and final proportions immature, initial (1848) population size, the minimal population, and the year in which it occurred (usually 1910 or 1912). The essentials (for $z = 5.04$) are summarized in Fig. 1 and 2. The solid lines are plotted from equation (7), showing maximum net recruitment

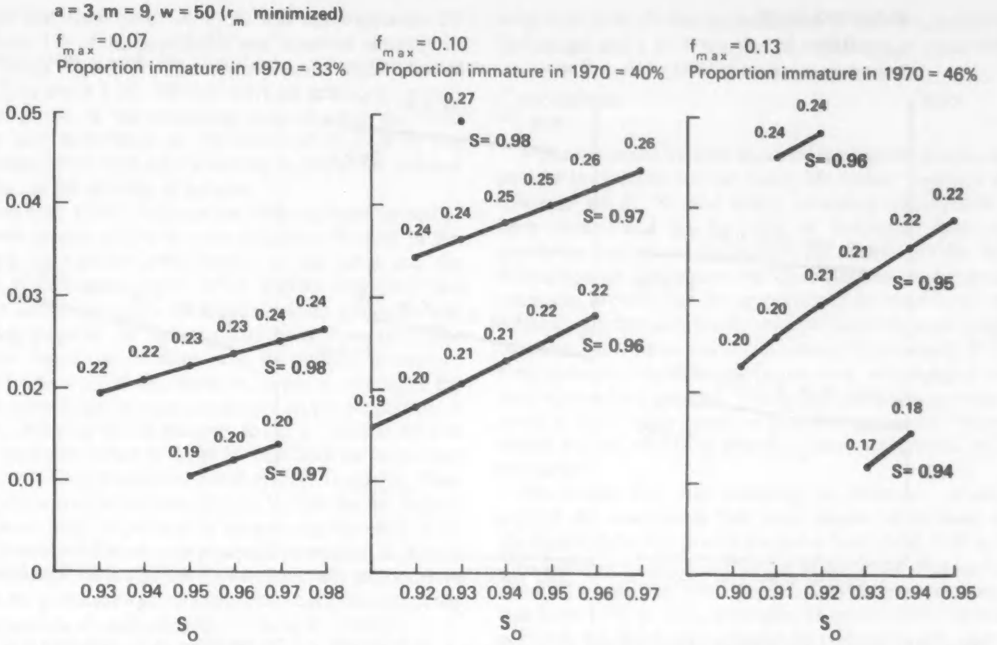

FIG. 1. Plots of r_m versus s_0 for different values of s and f_{max}, with a = 3, m = 9, and w = 50. Numbers above plotted lines represent the 1970 population size (4000) as a fraction of the estimated initial population size in 1848.

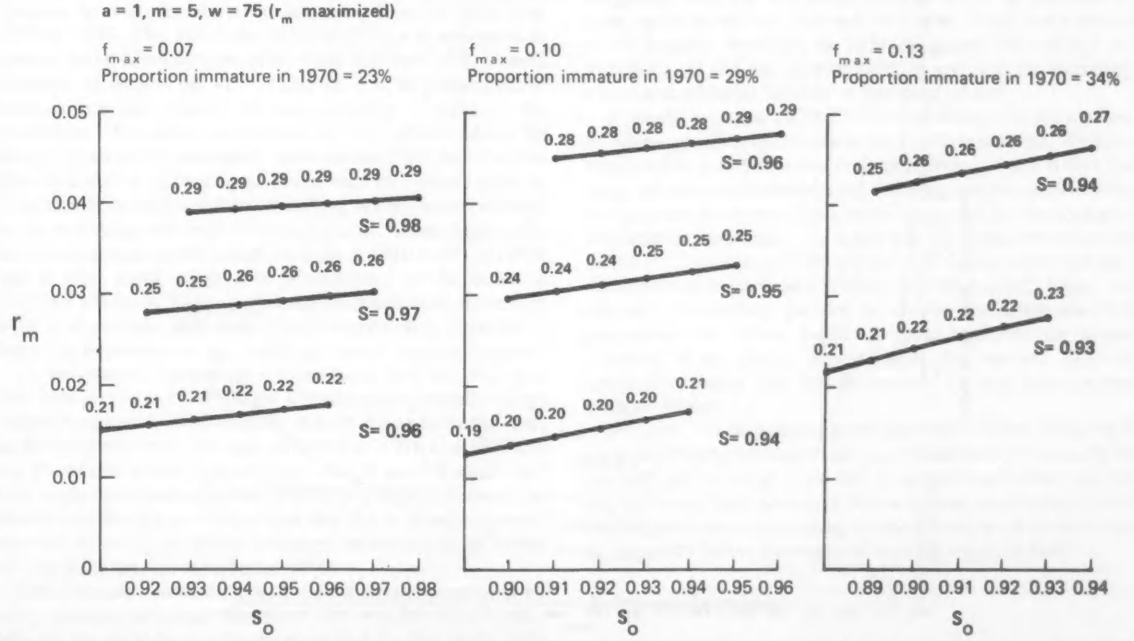

FIG. 2. Plots of r_m versus s_0 for different values of s and f_{max}, with a = 1, m = 5, and w = 75. Numbers above plotted lines represent the 1970 population size (4000) as a fraction of the estimated initial population size in 1848.

rates for values of s_0 and s that fall within the ranges studied at the three levels of f_{max} considered here.

As would be expected, higher survival rates are required to sustain the population at the lower values of productivity (f_{max}). One of the striking features of the plots is the very substantial change in r_m associated with a change in adult survival of one percentage point. This has been emphasized before (e.g. Eberhardt and Siniff 1977; Goodman 1981) but is especially important here, since it shows the uncertain nature of inferences about population trend based on rough notions of adult survival.

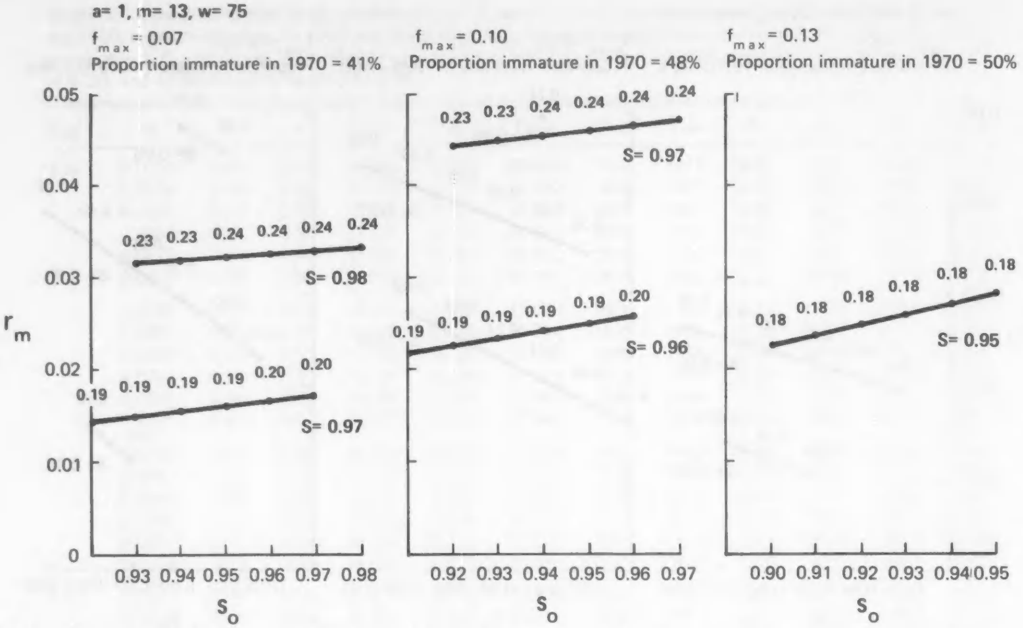

FIG. 3. Plots of r_m versus s_0 for different values of s and f_{max}, with $a = 1$, $m = 13$, and $w = 75$. Numbers above plotted lines represent the 1970 population size (4000) as a fraction of the estimated initial population size in 1848.

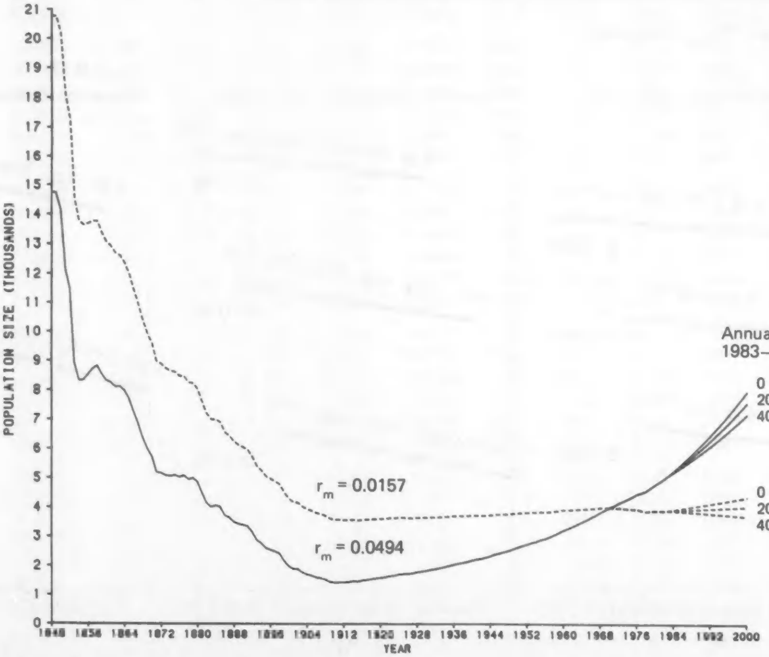

FIG. 4. Example of two sets of population trajectories from 1848 to 2000, for $a = 3$, $m = 9$, $w = 50$, and $f_{max} = 0.10$. Annual kills are applied from 1983 to 2000.

If the proportion of immatures (40%) reported by Johnson et al. (1981) is correct, then it appears that delayed maturity (i.e. at a late age) must be assumed. The other parameters in the central panel of Fig. 1 corresponding to a 40% proportion of immatures appear reasonable, in terms of estimates for other baleen whales, and the limited bowhead data (except, of course, that the assumed reproductive rate is well above many of the observed estimates). For the adult survival rate of 0.97, a 1910 population on the order of 2000 whales is obtained (Table 2). This then gives a rate of increase to 1970 of about 0.012. Since the average kill from 1910 to 1970 was approximately 22 whales, about 1% of the population was removed annually in the initial years after 1910. The corresponding eigenvalue of equation (2) is about 1.02. We thus have an indication, within very broad limits of the modeling exercise, that the 1970 population may have been on the order of 20–25% of the preexploitation level, with a net recruitment rate on the order of 2% per year, in the absence of harvest.

The Davis et al. (1983) findings are different from the earlier findings with respect to the fraction of calves (9–12% of the total, based on various arrangements of the data) and the proportion of immatures (about 60%). Further analysis of the preliminary data may narrow the range of values somewhat and perhaps indicate why the values differ from those of earlier studies. Not much can be done with the models to explore further the fraction of calves. We have, however, examined the effect of a delayed age of sexual maturity on the proportion of immatures. Delaying sexual maturity to age 13 (so that the first calves are born when the cow is age 14) increases the proportion of immatures in the population somewhat (Fig. 3), ranging from 41% for the lower reproductive rates up to 50% for the highest rate considered here. At present, it appears that the most likely explanation of the difference in observed proportion of immatures in the population is that there is a tendency for reproductive animals in the population to be somewhat segregated from the other components, as suggested by Davis et al. (1983).

Projections from 1970 to the Year 2000

Much of the recent research on bowheads was initiated in response to concerns about the increased level of takes from 1970 to 1977. The kill from 1910 to 1970 was estimated to average about 22 whales per year, while that from 1970 to 1982 averaged 38 whales per year (Table 1). It is thus important to examine possible impacts of this doubling of takes on the population. We cannot, of course, be very precise about the likely impact of the increased removals until the uncertainties described above are resolved. Some very elementary calculations may be considered first by looking at the ranges provided by the modeling outcomes (Tables 2 and 3). These suggest that the minimal population change from about 1910 or 1912 to 1970 was a very small increase in abundance, on the order of 200–300 whales in 60 yr. Increasing removals from an average of 22 to an average of 38 for a decade might thus be expected to lower the population in the 1980s by several hundred whales.

At the other extreme, populations from 1910 to 1912 may have been as low as 1300 whales. Consequently, annual average population gains to 1970 would be as high as 45 whales per year, so that probably only the high removals of 1976 and 1977 (56 and 32 whales killed, respectively, and 35 and 79 struck and lost) might have caused net losses to the population. It should be pointed out that these extremes are not due to density dependence but rather to parameter revisions necessary to get to the 1970 population size assumed at 4000.

These simple calculations may be elaborated somewhat by using selected parameter sets from Fig. 1 and 2 and carrying forward the projections already described for the 1848–1970 period. In these calculations, we assumed annual kills of 0, 20, and 40 from 1983 to the year 2000, and projected forward with a population size of 4000 in 1970. The corresponding year 2000 population sizes are given in the last three columns of Tables 2 and 3. An example of the population trajectories for the parameter combination $a = 3$, $m = 9$, $w = 50$, and $f_{\max} = 0.10$ is shown in Fig. 4. The two sets of trajectories correspond to the minimum (0.0157) and the maximum (0.0494) r_m for the above parameters and a 1970 fraction of immatures of about 40%.

Conclusions

While the model we have assumed is relatively simple, it does attempt to account for the basic life history features of the bowhead whale. Several rather sweeping assumptions have been necessitated by the lack of biological data on the population and by uncertainties in the records of take. Further difficulties are introduced by discrepancies in the available estimates, in particular, the proportion of immatures (40–60%), which is substantially higher than predicted by most modeling outcomes, and the proportion of calves (from roughly 3–12%). Until these discrepancies can be resolved, very definite conclusions cannot be supported. The figures and tables provided here cover a fairly wide range of possible parameter values and should permit readers to examine their own choices of likely outcomes.

The results from our modeling do, however, virtually all support the conclusion that some degree of increase in the population did occur from a low point from about 1910 to 1970. This increase occurred in the face of an annual average take of about 22 whales per year. Hence, it is unlikely that the increased take from 1970 to 1982, averaging 38 whales killed (estimated) per year, has drastically affected the population yet. Due to the many present uncertainties about relevant biological features of the population, it would be prudent to restrict future total kills to no greater than the kill levels prior to 1970, in the hope of assuring an increase in the stock. We believe that such a course is particularly desirable in light of future risks posed by increasing oil and gas developments in and near the migratory routes and essential habitats of bowhead whales.

It should be noted that different conclusions can be reached by adopting different choices of the basic parameters. We have attempted to maintain a relatively conservative view within the range of parameters established for other species while taking into account the range of estimates suggested by the data now available on bowheads. We doubt that the higher reproductive values ($f_{\max} > 0.07$) will be sustained by further investigations, since most of the estimates available are substantially lower. An especially interesting question is whether the lower observed proportions of calves might, in fact, represent the actual situation. If so, then it appears that adult survival must be appreciably higher than thus far reported for most other species of large whales.

We have not considered population trajectories based on a maximum net reproductive rate (r_m) of less than 0.01, mainly on grounds that we know of no data to support such a low rate for any species of large mammal. In our opinion, assuming a lower rate is tantamount to assuming that bowheads were on their way to extinction before commercial whaling began in 1848.

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Prediction of Chlorophyll *a* Concentrations in Florida Lakes: Importance of Aquatic Macrophytes[1]

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Chlorophyll *a* concentrations in Lake Pearl, Florida, increased as the percentage of the lake's total volume infested with aquatic macrophytes decreased. Using data from 32 Florida lakes having a wide range of limnological characteristics, we demonstrated that predictions of chlorophyll *a* concentrations could be improved by including a term for the percentage of the lake's total volume infested with macrophytes in existing nutrient–chlorophyll models. Our best-fit multivariate regression equation was

$$\log \mathrm{CHLA} = 1.02 \log \mathrm{TN} + 0.28 \log \mathrm{TP} - 0.005\ \mathrm{PVI} - 2.08$$

where CHLA is the chlorophyll *a* concentration (milligrams per cubic metre), TN is the total nitrogen concentration (milligrams per cubic metre), TP is the total phosphorus concentration (milligrams per cubic metre), and PVI is the percentage of the lake's total volume infested with macrophytes. By use of this equation, we assessed the potential effect of aquatic macrophytes on chlorophyll yields and Secchi disc transparencies in lakes of different trophic status.

Les concentrations de chlorophylle *a* dans le lac Pearl, en Floride, augmentent en fonction de la diminution du pourcentage du volume total du lac infesté par des macrophytes aquatiques. Nous démontrons, à partir de données sur 32 lacs de la Floride, à gammes étendues de caractéristiques limnologiques, que les prédictions de concentration de chlorophylle *a* pouvaient être améliorées en incluant dans les modèles existants de chlorophylle–nutriments un terme représentant le pourcentage du volume total du lac infesté par des macrophytes. L'équation de régression multidimensionnelle qui s'adapte le mieux est la suivante :

$$\log \mathrm{CHLA} = 1{,}02 \log \mathrm{TN} + 0{,}28 \log \mathrm{TP} - 0{,}005\ \mathrm{PVI} - 2{,}08$$

où CHLA est la concentration de chlorophylle *a* (milligrammes par mètre cube), TN la concentration d'azote total (milligrammes par mètre cube), TP la concentration de phosphore total (milligrammes par mètre cube) et PVI le pourcentage du volume total du lac infesté par des macrophytes. Cette équation nous permet d'évaluer l'effet possible des macrophytes aquatiques sur le rendement en chlorophylle et la transparence au disque de Secchi dans des lacs d'états trophiques différents.

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Studies of lakes located in different geographical regions have demonstrated that there is a strong correlation between nutrient (total phosphorus and total nitrogen) concentrations and algal biomass as measured by chlorophyll *a* concentrations (Sakamoto 1966; Smith 1982; Canfield 1983). Thus, nutrient–chlorophyll regression equations have gained acceptance as a tool for estimating the response of lakes to reductions or increases in nutrient loading rates (e.g. Dillon and Rigler 1975). Although these equations provide a basis for understanding differences in the algal biomass of lakes (Jones and Bachmann 1976), confidence limits for predicted chlorophyll concentrations are broad and range over an order of magnitude (Canfield 1983). This makes it difficult to obtain accurate predictions for specific lakes.

Explanations for the broad confidence limits include differences in analytical methods and time of sampling (Nicholls and Dillon 1978), zooplankton grazing (Shapiro 1979), and limitation by nonalgal turbidity (Hoyer and Jones 1983). Recently, there have been attempts to quantify the importance of these possible sources of variability in nutrient–chlorophyll regressions (Hoyer and Jones 1983). Numerous studies, however, have suggested that aquatic macrophytes, especially submersed macrophytes, can inhibit the development of phytoplankton (Hasler and Jones 1949; Hogetsu et al. 1960). Aquatic macrophytes could therefore be an important source of the variability in nutrient–chlorophyll regressions. Existing macrophyte studies, however, have provided no quantitative information on how different levels of macrophyte abundance influence chlorophyll concentrations in lakes. Our purpose in this paper is to examine the influence aquatic macrophytes have on chlorophyll yields in lakes and to determine the effect of this influence on nutrient–chlorophyll models.

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Methods

Lake Pearl (Table 1), a 24-ha eutrophic Florida lake (28°36′N, 81°16′W), was sampled monthly between February 1979 and April 1983 to determine changes in total phosphorus (TP), total nitrogen (TN), chlorophyll *a* (CHLA), macrophyte coverage (C), and the percentage of the lake's total volume infested with aquatic macrophytes (PVI). Lake Pearl has a maximum depth of 7 m and a mean depth of 2.5 m at normal water levels, but these characteristics changed significantly during our study when drought conditions reduced the lake volume by approximately 50%. Although a variety of macrophytes including southern naiad (*Najas guadalupensis*), Eurasian watermilfoil (*Myriophyllum spicatum*), spatterdock (*Nuphar luteum*), and fragrant water lily (*Nymphaea odorata*) were present, coverage was less than 10%. Hydrilla (*Hydrilla verticillata*), an exotic that was introduced in the mid-1970s, was the dominant macrophyte covering over 95% of the lake during periods of peak infestation.

Water samples were collected from 0.5 m below the surface and 0.3 m above the bottom at six stations located in different areas of the lake. Sampling stations included both limnetic and littoral areas when these areas were present. Samples were placed on ice and returned to the laboratory at Gainesville, Florida, and processed within 24 h. TP analyses (Murphy and Riley 1962) were done after a persulfate oxidation (Menzel and Corwin 1965). TN was determined by a modified Kjeldahl technique (Nelson and Sommers 1975). Water was filtered through Gelman type A-E glass fiber filters for CHLA determination. CHLA was determined by using the method of Yentsch and Menzel (1963) and the equations of Parson and Strickland (1963).

C and PVI were determined by use of a DE-719 (Raytheon Marine Co., Manchester, NH) survey fathometer (Maceina and Shireman 1980). Thirteen transects, which covered a total of 3.6 km, were used to measure macrophyte abundance. Macrophyte abundance in Lake Pearl was experimentally manipulated by using 11 herbicide treatments and three grass carp (*Ctenopharyngodon idella*) stockings. In late 1981 and early 1982, sufficient grass carp were stocked to eliminate all submersed vegetation.

To obtain data from a larger number of lakes, 32 lakes were sampled once in either 1981 or 1982 during the period of peak macrophyte abundance (June–October). Three lakes were sampled once in both 1981 and 1982; thus, we obtained 35 data observations for our data base. At each lake, surface water (0.5 m) samples were collected from three midlake stations and the fathometer was used to determine macrophyte abundance. The number of fathometer transects varied depending on lake size. Procedures used to determine TP, TN, CHLA, C, and PVI values were the same as for Lake Pearl.

Results and Discussion

C ranged from nearly 100 to 0% during the 4-yr study at Lake Pearl (Fig. 1). PVI ranged from 80 to 0% and CHLA ranged from 0.6 to 60 mg/m^3. Examination of the data (Fig. 1) showed a trend for increases in chlorophyll concentrations when large reductions in PVI occurred and a reduction in chlorophyll levels when PVI increased. Correlation analysis of the CHLA and PVI values collected during the study showed that there was a significant negative correlation ($r = -0.63$; $P < 0.01$). Based on these data, we hypothesized that variations in PVI could be a significant component of the variance in nutrient–chlorophyll regressions.

TABLE 1. Chemical conditions measured in Lake Pearl between February 1979 and November 1982.

Characteristic	Mean	Range
pH	7.1	6.1–10.5
Total alkalinity (mg/L as $CaCO_3$)	39	8–74
Specific conductance (μS/cm, 25°C)	165	16–270
Calcium (mg/L)	16	6.4–24
Magnesium (mg/L)	5.9	0.7–8.1
Total phosphorus (mg/m^3)	28	1–365
Total nitrogen (mg/m^3)	740	48–3760

We developed several regression models using the TP, TN, CHLA, and PVI data (Table 2) collected during our survey of 32 Florida lakes to determine if the addition of a PVI term could improve the predictive ability of nutrient–chlorophyll models. Although our sampling was limited, a wide range of limnological conditions were sampled. TP concentrations ranged from 0.6 to 159 mg/m^3 and TN concentrations ranged from 65 to 6020 mg/m^3. CHLA values ranged from 0.5 to 174 mg/m^3 and PVI levels ranged from 0 to 95%. Regression models for our data set were as follows:

$$(1)\quad \log \mathrm{CHLA} = 1.09 \log \mathrm{TP} - 0.40, \qquad R^2 = 0.73$$

$$(2)\quad \log \mathrm{CHLA} = 1.16 \log \mathrm{TN} - 2.24, \qquad R^2 = 0.78$$

$$(3)\quad \log \mathrm{CHLA} = 0.73 \log \mathrm{TN} + 0.51 \log \mathrm{TP} - 1.65, \qquad R^2 = 0.82$$

$$(4)\quad \log \mathrm{CHLA} = 1.02 \log \mathrm{TN} + 0.28 \log \mathrm{TP} - 0.005\ \mathrm{PVI} - 2.08, \qquad R^2 = 0.86.$$

For a larger sample of Florida lakes, Smith (1982) and Canfield (1983) found that R^2 values for their TP–TN regression models were 0.83 and 0.81, respectively. These values agree with the value determined for our data. Thus, our data are comparable with the larger data sets. By incorporating a PVI term (eq. 4), an additional 4% of the variance was accounted for and the regression coefficients for TP and TN remained similar to those reported by Canfield (1983). Although small, the 4% increase in R^2 was significant and indicates that the percent of a lake's total volume infested with aquatic macrophytes significantly influences chlorophyll concentrations in Florida lakes.

To test the predictive ability of our multiple regression model, we used eq. 4 to predict chlorophyll concentrations in Lake Pearl, as data from this lake were not used in developing the model. CHLA measured between February 1979 and April 1983 averaged 12 mg/m^3. Predicted CHLA (eq. 4) averaged 9 mg/m^3 and were not significantly different ($P < 0.05$) from the measured average. This suggests that the model is sensitive to average conditions. Generally good agreement also existed between measured and predicted seasonal values, but the model did not accurately predict all peak chlorophyll values (Fig. 2). The inability to predict peak chlorophyll values after herbicide treatments occurs because macrophytes release nutrients to the phytoplankton in the water before decomposition significantly reduces plant height. Other factors, however, may also be involved. For example, the high chlorophyll values observed after grass carp eliminated all submersed vegetation were transitory (Fig. 1); therefore, other modifications in the model may be necessary if precise seasonal predictions of chlorophyll yields are required.

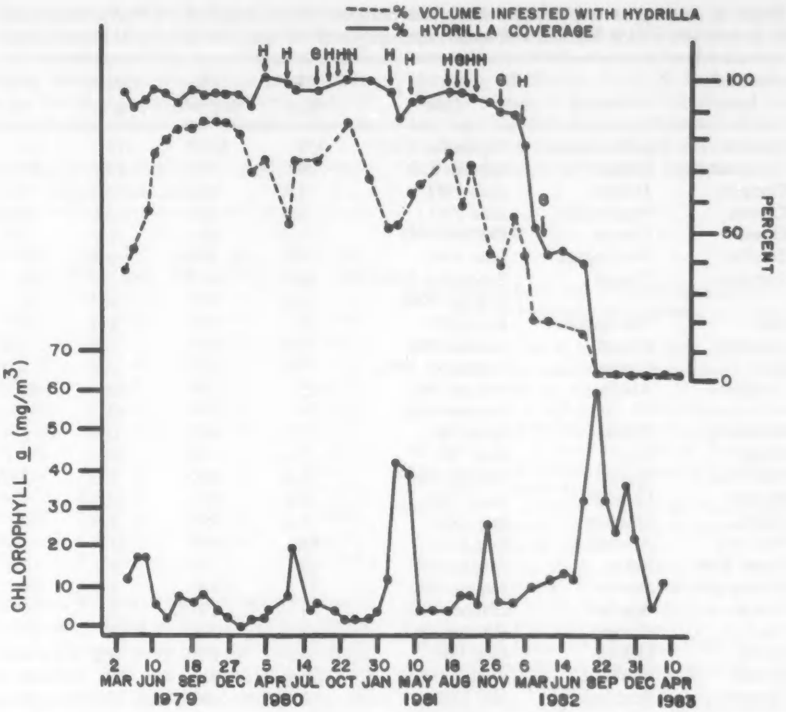

FIG. 1. Changes in chlorophyll *a* concentrations, macrophyte (hydrilla) coverage, and the percent of the lake's total volume infested measured in Lake Pearl. H = a herbicide treatment; G = a grass carp stocking.

To assess what the potential impact of different levels of macrophyte abundance might be on chlorophyll yields in lakes ranging from oligotrophic to eutrophic, we used eq. 4 to predict CHLA values for five hypothetical combinations of TP and TN values (assuming phosphorus limitation and TN:TP = 20:1) given PVI values ranging from 0 to 100% (Table 3). Our theoretical analysis demonstrates that a continuous decrease in CHLA occurs as PVI increases in lakes of all trophic status. The magnitude of the impact of increasing PVI, however, varies with trophic state. For a nutrient-poor lake such as case 1 (Table 3), the expected reduction in CHLA with increasing macrophyte abundance is small even if macrophytes could occupy 100% of the lake volume. The reduction would also be small enough that only an intensive sampling program would demonstrate statistically significant changes in CHLA values with changes in PVI. Major changes in Secchi disc transparencies, however, would occur with large changes in PVI. For lakes such as cases 2 and 3 (Table 3), the predicted declines in CHLA are great enough that even a less intensive sampling program would most likely demonstrate an impact of macrophytes, especially if PVI values changed from 0 to 100%. In these lake types, large changes in macrophyte abundance could also cause changes in trophic classifications if chlorophyll values were used as the trophic criterion. For nutrient-rich lakes (cases 4 and 5), the changes in CHLA are large enough that even an infrequent sampling program would most likely document a significant impact of macrophytes on lake chlorophyll levels. However, even at a PVI of 100%, there would be sufficient chlorophyll to classify a lake as eutrophic and to maintain reduced water transparency.

Although our analysis (Table 3) suggests that an increase in macrophyte abundance reduces lake chlorophyll levels, our results should not be used to suggest that macrophytes can be used to improve lake water quality by reducing chlorophyll concentrations. For all our hypothetical examples, major reductions in CHLA do not occur until PVI values exceed 30% (Table 3). Based on our experience at Lake Pearl and several other Florida lakes, PVI values in excess of 15% constitute a major weed problem for the public. If a reduction in macrophyte abundance is begun before PVI values exceed 15%, the effects of weed management on chlorophyll levels and Secchi disc transparencies should be minimal. However, if reductions begin after PVI values have obtained high levels and management objectives are to reduce weed infestations below problematic levels, noticeable increases in chlorophyll and reductions in water transparency (especially in lakes such as cases 2 and 3) will most likely occur. By informing the various user groups about these changes prior to initiation of weed management, many potential controversies over the impact of aquatic plant management can be avoided.

We are not certain what are the causative mechanisms for the inverse relationship between chlorophyll *a* concentrations and the percent of a lake's total volume infested with aquatic macrophytes. Several factors, however, are probably involved. These include (1) competition for nutrients by macrophytes and their associated epiphyton, (2) reduction in nutrient cycling because macrophytes reduce wind mixing and the resuspension of nutrients from the bottom sediments, and (3) increased sedimentation of planktonic algae due to a reduction in water

TABLE 2. Average total phosphorus (TP), total nitrogen (TN), chlorophyll *a* (CHLA), and percent of the total lake volume infested with aquatic macrophytes (PVI) values measured in 32 Florida lakes.

Lake	County	Date	TP (mg/m^3)	TN (mg/m^3)	CHLA (mg/m^3)	PVI (%)
Apopka	Lake/Orange	September 1981	159	6020	174	0
Catherine	Orange	October 1982	29	850	13	35
Compass	Jackson	June 1981	4.8	148	0.7	0.9
Crystal	Washington	June 1981	5.5	118	0.5	0.1
Down	Orange	October 1981	9.2	284	1.7	3
Dunford	Washington	June 1981	6.3	214	0.8	23
Fairview	Orange	September 1981	10	447	2.5	35
		October 1982	9.6	450	4.2	33
Gap	Washington	June 1981	10	285	1.4	15
Goodson	Putnam	August 1982	5.9	117	3.5	2.0
Kerr	Marion	September 1981	8.2	202	2.6	1.0
Lochloosa	Alachua	August 1981	25	1230	29	48
		October 1982	34	1530	40	35
McKenzie	Calhoun	June 1981	3.1	183	0.9	2.9
Merial	Bay	June 1981	5.7	65	0.6	3.1
Mill Dam	Marion	August 1982	8.8	439	7.0	7.4
Mirrow	Calhoun	June 1981	3.4	117	0.8	7.7
Mystic	Madison	July 1982	3.1	459	4.9	78
Newnans	Alachua	July 1982	68	1330	43	3.7
Ocean Pond	Baker	August 1982	19	376	5.1	0.1
Okahumpka	Sumter	August 1981	12	1330	4.1	95
Orange	Alachua	October 1982	24	850	12	79
Pineloch	Orange	October 1982	23	715	11	23
Round	Jackson	June 1981	4.8	173	0.9	0.8
Rowell	Bradford	August 1982	76	916	36	15
Sampson	Bradford	July 1982	13	480	3.3	11
		October 1982	25	672	3.1	42
Sellers	Marion/Lake	August 1982	2.3	266	3.3	1.2
Stella	Putnam	September 1981	12	490	1.6	39
Townsend	Lafayette	July 1981	5.5	527	4.6	65
Turkey Pen	Calhoun	June 1981	4.8	86	0.6	1.9
Tyler	Orange	October 1982	16	480	7.2	60
Watertown	Columbia	August 1982	42	1200	82	1.2
Wauberg	Alachua	October 1982	109	2200	140	0
Wildcat	Lake	August 1982	0.6	184	2.6	1.5

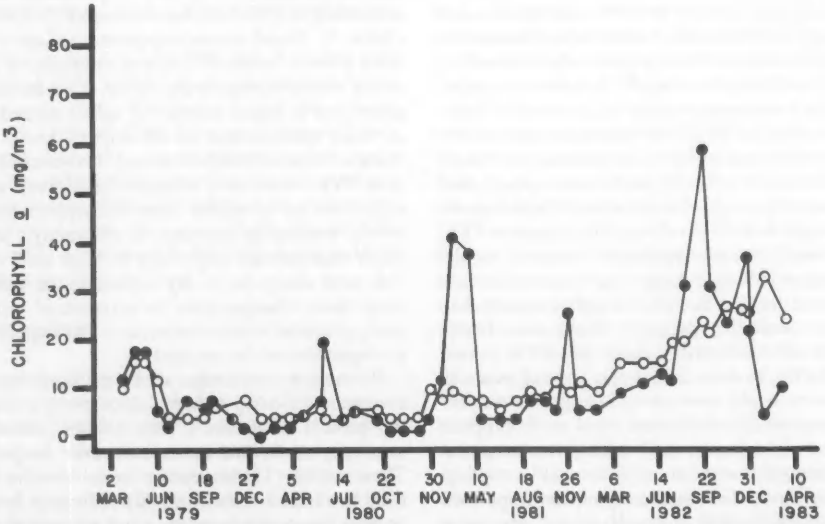

FIG. 2. Measured chlorophyll *a* concentrations (●) in Lake Pearl and chlorophyll concentrations calculated with eq. 4 (○).

TABLE 3. Chlorophyll *a* concentrations (mg/m^3) predicted by use of eq. 4 for five hypothetical cases that depict different combinations of total phosphorus (TP) and total nitrogen (TN) concentrations (mg/m^3) and percent of the total lake volume infested with macrophytes (PVI). Values in parentheses are Secchi disc transparencies (m) predicted using the Secchi–chlorophyll relationships of Jones and Bachmann (1978).

	Hypothetical case				
PVI (%)	1 (TP = 10, TN = 200)	2 (TP = 20, TN = 400)	3 (TP = 40, TN = 800)	4 (TP = 80, TN = 1600)	5 (TP = 160, TN = 3200)
0	3.5 (3.2)	8.7 (2.0)	21 (1.2)	53 (0.7)	129 (0.4)
10	3.1 (3.4)	7.8 (2.1)	19 (1.3)	47 (0.8)	115 (0.5)
20	2.8 (3.6)	6.9 (2.2)	17 (1.4)	42 (0.8)	102 (0.5)
30	2.5 (3.9)	6.2 (2.4)	15 (1.5)	37 (0.9)	91 (0.5)
40	2.2 (4.1)	5.5 (2.5)	13 (1.6)	33 (0.9)	81 (0.6)
50	2.0 (4.4)	4.9 (2.7)	12 (1.6)	30 (1.0)	72 (0.6)
60	1.8 (4.6)	4.4 (2.8)	11 (1.7)	26 (1.1)	65 (0.7)
70	1.6 (4.9)	3.9 (3.0)	10 (1.8)	23 (1.2)	58 (0.7)
80	1.4 (5.3)	3.5 (3.2)	8.5 (2.0)	21 (1.2)	51 (0.7)
90	1.3 (5.5)	3.1 (3.4)	7.6 (2.1)	19 (1.3)	46 (0.8)
100	1.1 (6.0)	2.8 (3.6)	6.8 (2.2)	17 (1.4)	41 (0.8)

turbulence by macrophytes. Whatever the mechanism may be, our analysis suggests that the percent of the lake's total volume infested with aquatic macrophytes may be a useful and easily obtainable empirical measure that can be used to assess the impact of aquatic macrophytes on lake chlorophyll levels. We must recognize, however, that other factor such as zooplankton grazing, limitation by nonalgal turbidity, and hydraulic flushing rates may be more important than the percent of a lake's volume infested with macrophytes in determining lake chlorophyll levels. Thus, the amount of variance explained by including a PVI term will depend on the lakes included in the data set. For example, Hoyer and Jones (1983) have shown that the concentration of inorganic sediments can explain much of the residual variance in turbid systems. In these lake types, it would be difficult to show an effect of aquatic macrophytes. A systematic evaluation of various factors contributing to the residual variance in nutrient–chlorophyll models is therefore needed. Our sampling was limited; hence, further testing is needed to ascertain the applicability of our results to other Florida lakes and to lakes located in other geographical regions.

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Small-Scale Covariability in the Abundance of Fish Larvae and Their Prey[1]

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Fortier, L., and W. C. Leggett. 1984. Small-scale covariability in the abundance of fish larvae and their prey. Can. J. Fish. Aquat. Sci. 41: 502–512.

We studied the covariability in the abundance of larval fish and microzooplankton at a fixed station of the St. Lawrence upper estuary. Hourly sampling during 129 h combined with net and tidal circulations allowed us to resolve spatial scales of approximately 2 km to several tens of kilometres. Low-frequency fluctuations in the abundance of larval capelin (*Mallotus villosus*) and larval Atlantic herring (*Clupea harengus harengus*) reflected the advection past the station of successive cohorts. Large-scale horizontal gradients in abundance and length were apparently formed at the emergence of the larvae from the spawning beds. The majority of capelin larvae we sampled had not yet completely exhausted their yolk reserves. There was no indication that differential mortality or active swimming had produced a concordance between the distribution of this species and the distribution of microzooplankton. Herring larvae resorbed their yolk sac and began mandatory exogenous feeding during the period of sampling. Tidal and subtidal patterns of variation in the abundance of this species and microzooplankton became significantly coherent after the resorption of the vitelline reserves. The possible mechanisms giving rise to coherence at these two different scales are considered in light of published information on the feeding behavior and swimming capacity of herring larvae.

La covariabilité entre l'abondance des stades larvaires de poisson et l'abondance du microzooplancton a été étudiée à une station fixe de l'estuaire moyen du Saint-Laurent. Un échantillonnage horaire maintenu pendant 129 h a permis, en combinaison avec la marée et la circulation résiduelle, de résoudre des échelles spatiales d'environ 2 km à plusieurs dizaines de kilomètres. Les fluctuations de basse fréquence dans l'abondance des larves de capelan (*Mallotus villosus*) et de hareng de l'Atlantique (*Clupea harengus harengus*) reflètent l'advection, à la station d'échantillonnage, de différentes cohortes. A grandes échelles, les gradients horizontaux d'abondance et de longueur sont apparemment formés lors de l'émergence des larves des frayères. La majeure partie des larves de capelan possédait encore des réserves vitellines au moment de l'échantillonnage. Aucun indice de mortalité différentielle ou d'un comportement natatoire pouvant conduire à une concordance entre la distribution de cette espèce et la destribution du microzooplancton n'a été décelé. Chez le hareng, la résorption complète des réserves vitellines et le début de la nutrition exogène obligatoire se sont produits au cours de l'échantillonnage. Après la résorption du sac vitellin, les patrons de variation dans l'abondance de cette espèce et du microzooplancton deviennent cohérents à l'échelle de la marée de même qu'à plus court terme. Divers mécanismes pouvant expliquer une concordance à ces deux échelles sont discutés en regard du comportement alimentaire et des capacités natatoires des stades larvaires de hareng.

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Recruitment to commercial stocks of *r*-selected fish species is believed to depend primarily on survival during the annual recolonization of the environment by the larval stage (e.g. Hempel 1965; Hunter 1976; Saville and Schnack 1981). Current hypotheses on the factors affecting fish survival during the first weeks of life invoke the availability of suitable food as the central determinant of future recruitment (Hjort 1914; Cushing 1972). Lasker (1975) suggested that stable aggregations of prey are necessary for the survival of the larvae, and Hunter (1976) emphasized the importance of assessing the scale and persistence of congregations of larval fish and their food in order to understand the processes governing starvation and mortality. Despite its often stated importance, knowledge of the small- and intermediate-scale spatial and temporal relationship between the distribution of larval fish and their prey is surprisingly limited (Sharp 1980; Theilacker and Dorsey 1980).

Small- and intermediate-scale correspondence in the distribution of fish larvae and their prey could result from differential starvation and mortality linked to local differences in food availability, or from active search by the larvae for higher prey concentration (Hunter 1976). Shelbourne (1957) provided evidence for the in situ occurrence of differential starvation by relating variations in the somatic condition of the larvae of plaice (*Pleuronectes platessa*) to differences in food supply. O'Connell (1980) also showed that the occurrence of starvation in the larvae of northern anchovy (*Engraulis mordax*) was contagious (patchy) and could be related to local minima in zooplankton abundance. The sampling resolution used in these studies was not sufficient, however, to estimate the spatial dimensions of these zones of starvation.

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Evidence has been given for the existence of a kinetic response of fish larvae to heterogeneity in the vertical distribution of their prey (Potter and Lough 1980; Ellertsen et al. 1981; Frank and Leggett 1982b, 1983a, 1983b; Fortier and Leggett 1983). Little is known about the response of the larvae to the distribution of their food in the horizontal plane. Hewitt's interesting study (1981) of the evolution of horizontal aggregation in northern anchovy and jack mackerel (*Trachurus symmetricus*) larvae showed that patchiness in the initial distribution of these species decreased during the first days of passive dispersal and then increased again as the swimming ability of the larvae developed. This result suggests that active swimming plays an important role in the development of horizontal aggregations. The index used by Hewitt (1981) and Houde and Lovdal (1982) to estimate the level of contagion in the distribution of the larvae measured the intensity of the pattern (the average amplitude of the variations) but not the scale or grain of that pattern (the actual spatial dimensions of the aggregations).

Few works describing the geometry of ichthyoplankton distribution at spatial scales of a kilometre to a few kilometres or at time scales of a few hours to days can be found in the literature. Colton et al. (1980) published a preliminary examination of larval herring abundance based on samples collected hourly over a period of 96 h in the area of Georges Bank. Densities were shown to fluctuate from 10.3 to 290.4/100 m^3 but no attempt was made to describe and quantify the pattern of the short-term fluctuations observed. Several time series of larval abundance have been recorded in estuaries (Lewis and Wilkens 1971; Lewis and Mann 1971; Pearcy and Myers 1974; Eldridge 1977; Fortier and Leggett 1982). Tidal advection of horizontal gradients in abundance explained most of the observed variability but no attempt was made to relate these gradients in larval fish abundance to gradients in prey abundance.

The verification of the present paradigm of larval fish mortality, which proposes that survival depends on the availability of suitable food, is withheld by a general lack of information on the horizontal distributions of the larvae and their prey at small- and intermediate-scales (hundreds of metres to a few kilometres). The aim of this study was to test the hypothesis that patterns of aggregation in the horizontal distribution of fish larvae in the St. Lawrence estuary relate to the distribution of microzooplankton.

Materials and Methods

Area Studied and Sampling

In early summer the St. Lawrence upper estuary (Fig. 1) is partially mixed. The vertical gradient of salinity is about 10‰ from surface to bottom. The general circulation in the area is cyclonic with a net penetration of water in the North Channel (Fig. 1) and a net extrusion along the south shore (d'Anglejan and Smith 1973). Net longitudinal transport is seaward in the diluted surface layer and landward in the deep saline layer. The amplitude of the semidiurnal tide varies from 2 to 5 m depending on the neap–spring cycle. The length of the semidiurnal tidal excursion is in the order of 10–20 km (Gagnon and Lacroix 1981; Fortier and Leggett 1983).

In June 1980, 193-h time series of larval fish and microzooplankton abundance in the 0- to 10-m, 10- to 20-m, and 20- to 40-m depth-strata were obtained at station 80-1 (Fig. 1). Maximum depth at high tide was 51 m. Samples were collected every 3 h during the first 63 h of the time series and then every hour for the remaining 129 h. Details of sampling procedures and gear efficiency are fully described in Fortier and Leggett (1983). Briefly, samples were taken from the bow of an anchored ship with a 50-cm standard net (80 µm mesh) equipped with an opening and closing device, a flowmeter, and a time–depth recorder. The net was repeatedly moved up and down within the limits of the desired depth-stratum at a speed of 0.5 m/s. With tidal current acting as the main filtration force, this procedure resulted in a multiple oblique pathway of the net through the selected depth interval. Estimates of larval fish abundance and length–frequency distribution obtained simultaneously with the standard net and a bongo sampler towed at 3.5 knots (6.5 km/h) within 1.5 km of the anchored ship compared well. No relationship could be demonstrated between current speed, which contributed to the filtration pressure, and the efficiency of the standard net (Fortier and Leggett 1983).

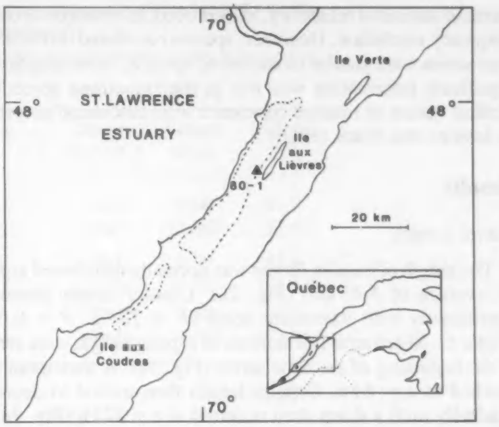

FIG. 1. Sampling location in the St. Lawrence estuary. The broken line represents the 50-m isobath, which roughly delineates the North Channel.

Temperature, conductivity, speed, and direction of currents were recorded at depths of 3, 10, 20, 30, 40, and 50 m with Aanderaa current meters. Hourly vertical profiles of the same variables, at depth intervals of 5 m, were obtained with a seventh meter.

Plankton samples were preserved in 4% formalin. From each sample, a maximum of 30 capelin and herring larvae were randomly selected and measured to the nearest 0.04 mm using the photographic technique of Zwanenburg (1982). Microzooplankton were enumerated with a model TaII Coulter counter for the following spherical-diameter intervals (in micrometres): 64–80, 80–102, 102–128, 128–160, 160–204, 204–256, 256–320, 320–408, 408–512, and 512–1024.

Analysis

Cross-correlation and spectral analyses require that the samples be taken at equal intervals in time and, hence, were applied to the hourly section of the time series only. The critical thresholds presented on the correlogram were computed according to Cox and Lewis (1966). Spectral estimates were obtained by standard expansions of the autocovariance function of the time series (routine FTFREQ of the International Mathematical and Statistical Library (IMSL)). The spectra were smoothed to increase statistical reliability. This procedure resulted in coarser frequency resolution. However, spectra calculated on the entire time series were similar to smoothed spectra, indicating that no significant information was lost in the smoothing procedure. Critical values of squared coherence were calculated according to Jenkins and Watts (1968).

Results

Larval Length

The length of capelin larvae was normally distributed around an average of 5.45 mm (Fig. 2a). Capelin length decreased significantly with increasing depth ($F = 253.2$, $P < 0.001$) (Table 1). Short-term fluctuations in capelin length were erratic at the beginning of the time series (Fig. 3a). A maximum was reached at $t = 61$ h. Capelin length then tended to decrease gradually until a sharp drop occurred at $t = 122$ h (Fig. 3a). It subsequently oscillated around an average level of 5.2 mm. No clear short-term pattern could be detected in the fluctuations with a sampling interval of 3 h ($t = 0$ to $t = 64$ h). During the hourly sampling ($t = 64$ h to $t = 193$ h), a 12.5-h tidal cycle, superimposed on the trend, was apparent (Fig. 3a). The percentage of yolk sac larvae also decreased significantly with depth (Wilson ANOVA: $\chi^2 = 48.1$, $P < 0.001$) (Table 1). This percentage remained high from $t = 0$ to $t = 65$ h and then dropped to an average level of 54% from $t = 65$ h to $t = 135$ h (Fig. 3b). Subsequently, the proportion of yolk sac larvae increased again (Fig. 3b).

The average length of herring larvae was 8.95 mm. The length–frequency distribution was slightly skewed toward smaller values ($g_1 = -0.59$, $P < 0.001$) (Fig. 2b). The length of herring larvae decreased significantly with depth ($F = 21.6$, $P < 0.001$) but in a less striking manner than capelin length. There was no discernible tidal or circadian pattern in the short-term fluctuations of larval herring length (Fig. 3c). Length tended to increase until a maximum was reached around $t = 101$ h. It then remained quite stable at an average level of 9.1 mm (Fig. 3c). The percentage of herring larvae with yolk sac did not vary significantly with depth (Wilson ANOVA: $\chi^2 = 4.58$, $P = 0.10$) (Table 1). The proportion with yolk sac remained high until $t = 60$ h and then progressively declined to less than 25% by the end of the sampling (Fig. 3d).

The $t = 64$ h to $t = 193$ h (hourly) portion of the time series of capelin length was filtered to remove the decreasing trend. The residuals (Fig. 4a) were compared with the time series of temperature at 10 m that was already stationary (Fig. 4b). Cross-correlation analysis showed that maximum temperature preceded maximum length of capelin larvae by 2 h (Fig. 5). An identical analysis revealed no significant relationship between the length of herring larvae and temperature ($r_{\max} = 0.115$, $P = 0.19$).

Larval Abundance

The overall average abundance of capelin and herring larvae was 642.8/100 m^3 and 50.3/100 m^3, respectively. Capelin larvae were concentrated near the surface, while herring larvae were more uniformly distributed over the water column (Table 1).

Capelin and herring larvae were classified into four length-categories (capelin: <5.0, 5.0–5.5, 5.5–6.0, and >6.0 mm; herring: <8.0, 8.0–9.0, 9.0–10.0, and >10.0 mm). The median categories (e.g. 5.0–5.5 and 5.5–6.0 mm for capelin)

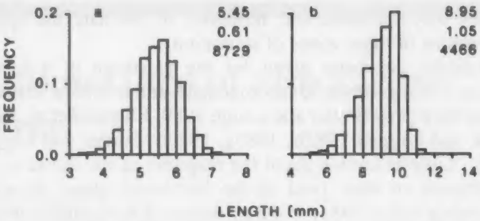

FIG. 2. Length–frequency distribution of (a) capelin and (b) herring larvae. The mean, the standard deviation, and the number of larvae measured are given.

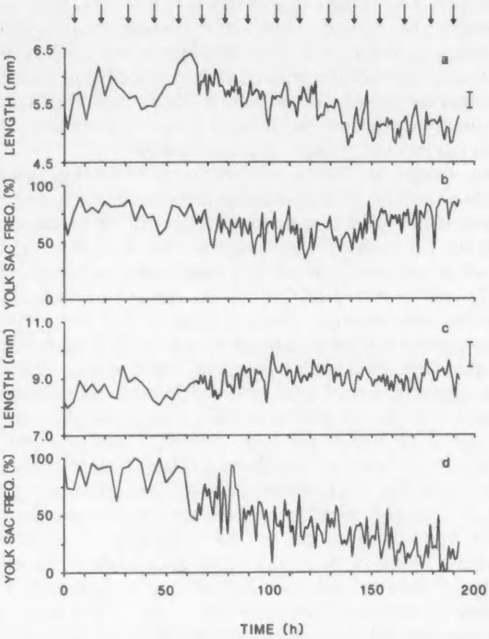

FIG. 3. Time series of (a) capelin average length, (b) capelin yolk sac frequency, (c) herring average length, and (d) herring yolk sac frequency. Arrows indicate maximum ebb. The vertical bar at the end of the curve in Fig. 3a and 3c corresponds to the average 5% confidence interval on the estimation of length.

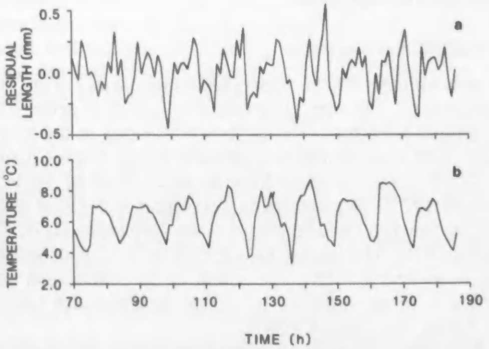

FIG. 4. Time series of (a) detrended capelin length and (b) temperature at 10 m.

TABLE 1. Average abundance, average length, and percentage of yolk sac larvae by depth-stratum ($n = 151$) (standard deviation given in parentheses).

	Capelin			Herring		
Depth-stratum (m)	Density (no./m^3)	Length (mm)	Yolk sac (%)	Density (no./m^3)	Length (mm)	Yolk sac (%)
0–10	1397.6 (1110.4)	5.58 (0.58)	69.6 (14.4)	55.2 (50.0)	9.08 (1.06)	41.7 (31.6)
10–20	473.6 (713.8)	5.36 (0.62)	61.6 (17.7)	58.7 (52.5)	8.90 (1.03)	45.4 (33.1)
10–40	67.2 (166.4)	5.19 (0.61)	50.8 (27.4)	37.1 (49.5)	8.84 (1.03)	38.3 (30.3)

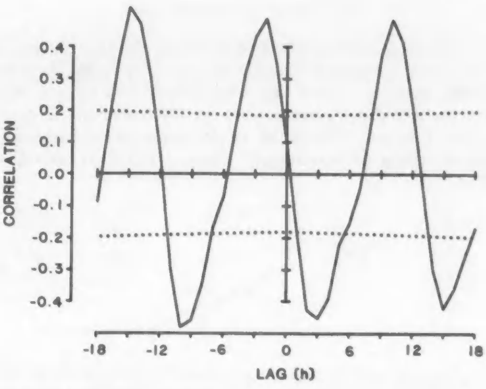

FIG. 5. Cross-correlation function between detrended capelin length and temperature at 10 m ($n = 117$). Dotted lines indicate the significance threshold for a probability of 5%.

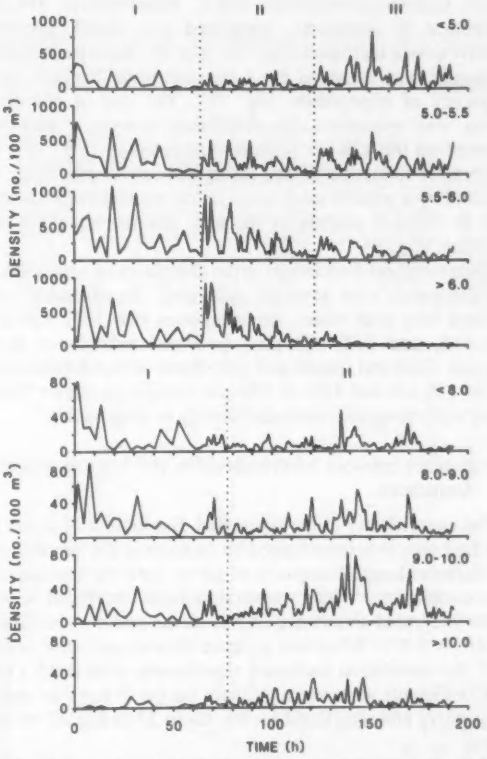

FIG. 6. Time series of larval capelin (top panel) and larval herring (bottom panel) abundance by length-category (mm). The different cohorts described in the text are delimited by vertical dotted lines and identified by roman numeral.

corresponded to approximately one standard deviation on each side of the respective average length of each species (Fig. 2). For each category, larval densities were integrated over depth so that the observed temporal fluctuations in abundance reflect the advection at the sampling station of horizontal discontinuities.

The long-term trend in capelin abundance suggested that three distinct cohorts of larvae were successively advected to the station (Fig. 6). The abundance was relatively high at the beginning of the sampling and progressively declined until t = 65 h when a second peak in density occurred in all length-categories, indicating the advection of a new cohort. Again, the abundance of this second cohort decreased progressively until t = 122 h when a third cohort appeared. The repartition of capelin larvae among the four length-categories was approximately the same for the first two cohorts, the larger length-categories being more abundant than the small length-categories (Fig. 6). This situation reversed with the advection of the third cohort, which consisted of smaller individuals. Cross-correlation analysis indicated that short-term fluctuations in capelin abundance closely followed fluctuations in temperature (Table 2). These fluctuations in temperature reflect tidal advection of the estuarine gradient in hydrographical conditions at the sampling station.

Variations in the abundance of herring larvae followed the same general trend in the four length-categories (Fig. 6). Abundances generally decreased from $t = 0$ to $t = 75$ h, suggesting that sampling began while a first cohort was being transported away from the station. Abundances increased from $t = 75$ h to $t = 140$ h and finally decreased again, as a second cohort was advected past the station (Fig. 6). Short-term fluctuations in herring abundance lagged fluctuations in temperature by 4 h except for larvae larger than 10 mm, which exhibited no significant cross-correlation with temperature (Table 2).

Microzooplankton Abundance, Composition, and Size

Microzooplankton was numerically dominated by nauplii of copepods, polychaete larvae, and copepodids (mainly *Acartia*

TABLE 2. Maximum cross-correlation (r_{max}) and lag between detrended larval fish abundance and temperature by length-category ($n = 117$). A negative lag indicates that maxima in abundance preceded maxima in temperature. $^{**}P < 0.01$.

Capelin			Herring		
Length category (mm)	r_{max}	Lag (h)	Length category (mm)	r_{max}	Lag (h)
<5.0	0.34**	−1	<8.0	0.22**	4
5.0–5.5	0.42**	0	8.0–9.0	0.37**	4
5.5–6.0	0.47**	1	9.0–10.0	0.25**	4
>6.0	0.37**	1	>10.0	0.12	—

clausi, *Calanus finmarchicus* and C. hyperboreus). The total abundance of organisms, integrated over depth, decreased steadily as size increased (Fig. 7a). The decline in the frequency of nauplii was balanced by a corresponding increase in the frequency of copepodids (Fig. 7b). The size of polychaete larvae was symmetrically distributed around a maximum centered on the 128- to 160-μm size-category (Fig. 7b). The progressive reduction of this taxon with increasing depth was reflected by a parallel attenuation of the crest characterizing the 100- to 200-μm portion of the total microzooplankton size-spectrum (Fig. 8).

Since temporal fluctuations in the abundance of some adjacent size-categories were strongly correlated, the organisms were grouped into four wider size-categories (64–128, 128–256, 256–512, and 512–1024 μm) to avoid redundancy in the analysis. Copepod nauplii and polychaete larvae dominated the 64- to 128-μm and 128- to 256-μm categories, while the two larger size-categories consisted mainly of copepodids.

Covariability between Ichthyoplankton and Microzooplankton Abundance

The covariability in the horizontal distribution of larval fish and their prey was investigated by correlating the abundance of the different length-categories of larvae with the abundance of microzooplankton. There was no significant correlation between depth-integrated abundance of fish larvae and microzooplankton ($P \geqslant 0.17$). When the separate depth-strata were considered, the correlation increased significantly with depth (Table 3). Coefficients were nonsignificant for the 0- to 10-m stratum but positive and significant for the 10- to 20-m and 20- to 40-m strata.

The relationship between larval fish and microzooplankton abundance also varied according to the size of microzooplankton. For example, in the 20- to 40-m stratum, capelin abundance was significantly correlated with the abundance of the smaller size-categories of microzooplankton only (64–128 and 128–256 μm) (Table 4). Herring larvae were significantly correlated with all size-categories of microzooplankton except the largest category (508–1024 μm) (Table 4). Similar results were obtained for the 10- to 20-m stratum where ichthyoplankton and microzooplankton abundance were also correlated (Table 3).

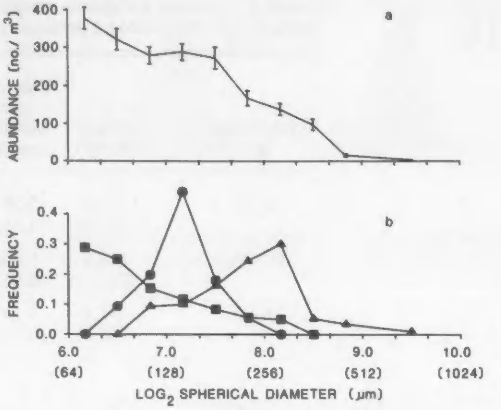

FIG. 7. (a) Total microzooplankton abundance by size-category; (b) size–frequency distribution of dominant taxa: nauplii (■), polychaete larvae (●), and copepodites (▲). Confidence limits on total microzooplankton abundance correspond to the 5% probability level ($n = 151$). Size–frequency distribution of dominant taxa is based on the exhaustive sorting of nine samples taken at 00:00 on each day of sampling.

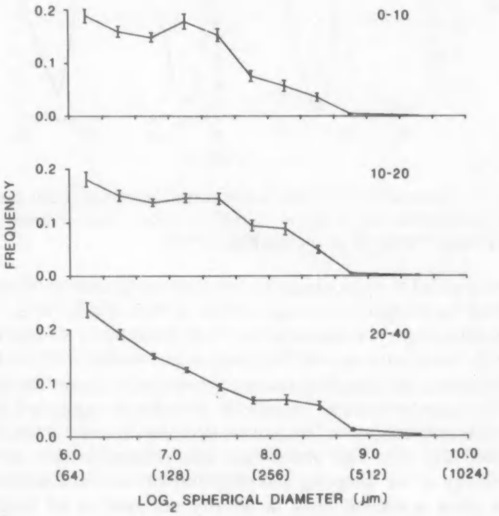

FIG. 8. Size–frequency distribution of total microzooplankton in the 0- to 10-m, 10- to 20-m, and 20- to 40-m depth-strata.

The time series of larval fish and microzooplankton abundance were submitted to spectral analysis to determine the scales at which significant correspondence existed in the distribution of the two taxa. Coherence and phase spectra were computed for each depth-stratum and for the entire water column. Coherence is a measure of the covariability between two signals at a given frequency or time scale, whereas phase is a measure of the synchronicity between two signals. For example, a significant coherence between two signals (e.g. the respective abundance of two taxa) at a given frequency indicates that fluctuations in the two variables at the time scale corresponding to this frequency are similar. A phase of 0° between the two signals at the same frequency would indicate that the fluctuations, in addition to being similar (coherent), are also synchronous and, hence, positively correlated. A significant coherence associated with a phase of 180° indicates a negative correlation between the two signals. Phases different from 0 and 180° denote the absence of synchronicity and, hence, a lack of direct dependence between the two variables. The spectra are obtained by plotting coherence or phase as a function of frequency.

TABLE 3. Correlation (r) between ichthyoplankton abundance and total microzooplankton abundance by depth-stratum and larval length-category ($n = 151$). Variables were log-transformed. The χ^2 statistic for the homogeneity of the correlation coefficients between the three depth-strata and the probability of observing a larger value are given. $^*P < 0.05$; $^{**}P < 0.01$.

Depth-stratum (m)	Capelin					Herring				
	<5.0 mm	5.0–5.5 mm	5.5–6.0 mm	>6.0 mm	Total	<8.0 mm	8.0–9.0 mm	9.0–10.0 mm	>10.0 mm	Total
0–10	−0.02	0.08	−0.02	−0.03	0.03	−0.02	−0.12	−0.07	−0.10	−0.08
10–20	0.26**	0.18*	0.16*	0.14	0.19*	0.08	0.23**	0.30**	0.31**	0.33**
20–40	0.29**	0.39**	0.25**	0.31**	0.37**	0.24**	0.40**	0.41**	0.37**	0.50**
χ^2	9.1	8.5	5.8	9.1	9.5	5.3	22.6	20.5	15.8	30.5
P	0.01	0.01	0.06	0.01	0.01	0.07	0.01	0.01	0.01	0.01

TABLE 4. Correlation (r) between ichthyoplankton abundance and microzooplankton abundance by size-category for the 20- to 40-m stratum ($n = 151$). Variables were log-transformed. $^*P < 0.05$; $^{**}P < 0.01$.

Size category (μm)	Capelin					Herring				
	<5.0 mm	5.0–5.5 mm	5.5–6.0 mm	>6.0 mm	Total	<8.0 mm	8.0–9.0 mm	9.0–10.0 mm	>10.0 mm	Total
64–128	0.22**	0.31**	0.22**	0.23**	0.33**	0.24**	0.39**	0.38**	0.24**	0.46**
128–256	0.16*	0.27**	0.21**	0.27**	0.30**	0.25**	0.39**	0.40**	0.27**	0.50**
256–512	0.04	0.11	0.06	0.12	0.11	0.16*	0.28**	0.35**	0.24**	0.42**
512–1024	0.04	0.13	0.17*	0.19*	0.15	0.01	−0.10	−0.11	−0.05	0.02

The general picture produced by the spectral analysis of the time series was similar to that obtained by the correlation approach, with no significant coherence between the depth-integrated abundance of larval fish and microzooplankton and a general increase in the coherence levels with depth. The coherence spectra revealed, however, that the frequencies or scales at which fluctuations in larval fish and microzooplankton abundance were correlated differed for capelin and herring.

Capelin

The shape of the coherence spectrum between larval capelin and microzooplankton abundance was similar for the three depth-strata. Significant coherence occurred in the frequency band corresponding to large-scale (36–18 h) and tidal (12–6 h) processes only. For example, in the 0- to 10-m depth-stratum that contained the bulk of the population, fluctuations in larval capelin and microzooplankton abundance were coherent for frequencies ranging from 0.04 to 0.22 cycles per hour (cph) (Fig. 9a). This indicates that the large-scale trends and tidal fluctuations in the abundance of the two taxa presented a similar pattern or grain. The phase spectrum revealed, however, that only the low-frequency fluctuations were simultaneous (phase ≃ 0°), tidal variations being out of phase (phase ≃ 270°) (Fig. 9a). An inspection of the time series confirms that the pattern or grain in the distribution of the two taxa was similar (coherent) and dominated by semidiurnal tidal variability (Fig. 10a and 10b). These tidal fluctuations, however, were not synchronous and only the relatively weak long-term trends coincide (Fig. 10a and 10b).

Herring

The shape of the coherence spectrum betwen larval herring and microzooplankton abundance was also similar for the three

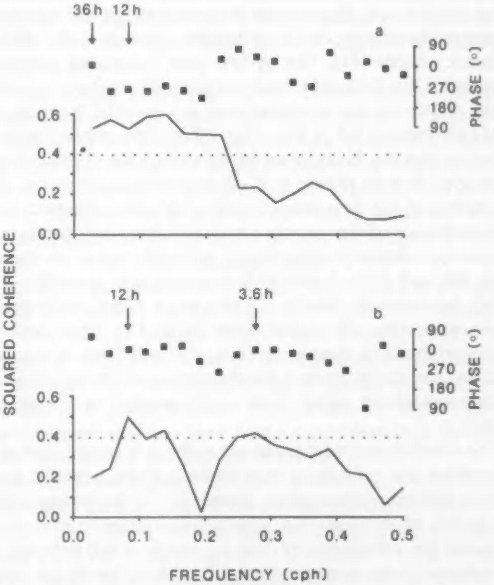

FIG. 9. Coherence (—) and phase (■) sprectra between (a) larval capelin and microzooplankton abundance in the 0- to 10-m depth-stratum and (b) larval herring and microzooplankton abundance in the 20- to 40-m depth-stratum. The dotted line represents the 95% confidence limit for nonzero squared coherence. Coherence is a measure of the covariability between two signals at a given frequency or scale, whereas phase is a measure of the synchronicity between two signals.

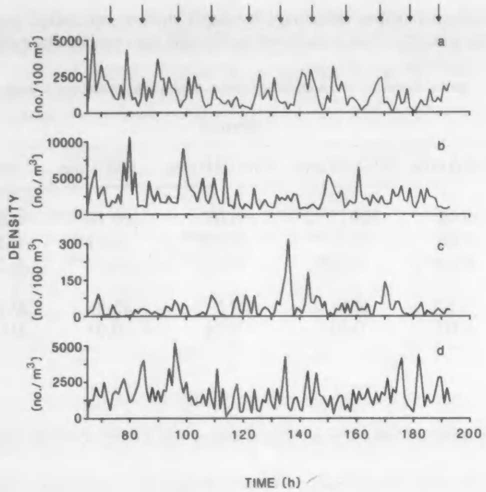

FIG. 10. Time series of (a) larval capelin and (b) microzooplankton abundance in the 0- to 10-m depth-stratum and time series of (c) larval herring and (d) microzooplankton abundance in the 20- to 40-m depth-stratum. Vertical arrows indicate maximum ebb.

depth-strata. However, coherence levels, like correlation coefficients (Table 3), increased with depth, being nonsignificant in the 0- to 10-m depth-stratum and reaching maximum values in the 20- to 40-m depth-stratum. In the 10- to 20-m and 20- to 40-m depth strata, fluctuations in larval herring and microzooplankton abundance were significantly coherent in two distinct frequency bands (Fig. 9b). A first peak in squared coherence occurred in the frequency band corresponding to tidal processes (0.08–0.14 cph) and a second peak at a subtidal frequency of 0.28 cph (period of 3.6 h) (Fig. 9b). The phase spectrum indicated that the fluctuations in the abundance of the two taxa were synchronous (phase ≃ 0°) at these frequencies (Fig. 9b). Inspection of the time series confirms that tidal fluctuations in the abundance of the two taxa were simultaneous, herring and microzooplankton densities being generally higher during ebb (Fig. 10c and 10d). Subtidal fluctuations also generally coincided. For example, from $t = 114$ h to $t = 126$ h, three distinct peaks in herring abundance corresponded to three peaks in microzooplankton abundance (Fig. 10c and 10d). Thus, both tidal and subtidal patterns in the abundance of herring larvae and microzooplankton significantly corresponded in the deepest strata.

The development of this correspondence was investigated by computing the coherence and phase spectra between larval herring and microzooplankton abundance for increasing length-categories of herring. No significant coherence was found between the abundance of herring larvae <8.0 mm and the abundance of microzooplankton (Fig. 11a). Significant coherence at frequencies corresponding to periods of a few hours (0.25–0.28 cph) first appeared in the spectrum of the 8.0- to 9.0-mm length-category and further developed in the spectra of the larger length-categories (Fig. 11a, 11b, and 11d). Significant coherence at the scale of the semidiurnal tide (0.08 cph) appeared later in the development of the larvae than subtidal coherence (Fig. 11).

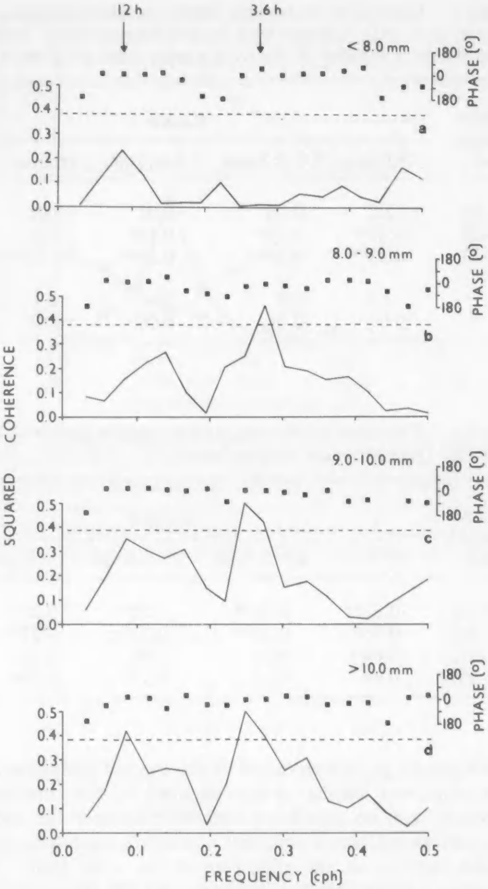

FIG. 11. Coherence (—) and phase (■) spectra between larval herring and microzooplankton abundance in the 20- to 40-m depth stratum for increasing length-categories of herring larvae. The dotted line represents the 95% confidence limit for nonzero squared coherence.

Discussion

Horizontal Gradients in Larval Fish Abundance

Capelin spawn on beaches and tidal flats on both shores of the St. Lawrence estuary (Parent and Brunel 1976). Herring deposit demersal eggs in the area of Ile Verte (Tremblay 1942). Trends in the time series of depth-integrated larval fish abundance (Fig. 6) reflect the advection, by residual circulation, of successive cohorts and suggest that emergence of the larvae from the spawning beds of the respective species is sporadic. Intermittent emergence is characteristic of capelin spawned on the beaches of eastern Newfoundland (Frank and Leggett 1981). In the St. Lawrence estuary, the large-scale spatial distribution of a given cohort forms a horizontal gradient that apparently exceeds the length of the tidal excursion: 10–20 km (Gagnon and Lacroix 1981; Fortier and Leggett 1983). Spatial gradients of 3 to 4 orders of magnitude extending over several tens of kilometres have been previously described in the distribution of the larvae of capelin (Jacquaz et al. 1977) and Atlantic herring (Able 1978) in the St. Lawrence estuary.

The semidiurnal tidal oscillation of the gradient formed by a given cohort results in 12.5-h modulations that become superimposed on the large-scale trends (Fig. 6). The occurrence of tidal fluctuations in capelin abundance, in phase or slightly out of phase with fluctuations in temperature (Table 4), initially suggests that longitudinal gradients in larval density may be formed by temperature-dependent differential survival. However, similar fluctuations in capelin abundance that were partially or totally out of phase with temperature fluctuations were reported by Fortier and Leggett (1982). Similarly, herring abundance, and temperature were out of phase by 4 h in the present study whereas a 6-h lag (inverse correlation) was observed earlier between the two variables (Fortier and Leggett 1982). These very different results, obtained for the same species in the same region in subsequent years, indicate that the formation of horizontal gradients in the abundance of ichthyoplankton is independent of the physical characteristics of the water masses occupied by the larvae.

Horizontal Gradients in Larval Length

The effect of temperature on the growth rates of larval fish has often been reported in the literature. Frank and Leggett (1982a) have shown that both growth rate and growth efficiency of larval capelin are influenced by temperature. In the present study, no simple relationship linked water temperature and capelin length. There existed longitudinal gradients in the horizontal distribution of both variables but these gradients were out of phase by 2 h and the length of capelin larvae was not monotonically proportional to the temperature of the surrounding water. We suggest, therefore, that length-gradients might also be formed at the emergence of the larvae from the spawning beds. The potential for environmentally induced differences in capelin length at emergence has been documented by Frank and Leggett (1982a).

Covariability in the Distribution of Larval Fish and its Prey

Significant covariability existed between the fluctuations in ichthyoplankton and microzooplankton biomass.The magnitude of this covariability differed with species, depth, larval fish size, and the spatiotemporal scale considered.

Capelin

The patterns of fluctuation in the abundance of capelin larvae and microzooplankton were similar but asynchronous except for the coincidence of the weak low-frequency variations. This result indicates that while the respective distributions of the two taxa were probably affected by the same turbulent processes, no direct dependence linked the local abundance of larval capelin to the abundance of microzooplankton. This finding is consistent with the results of earlier studies on newly hatched capelin. Courtois et al. (1982), using principal component analysis, found no correspondence between the distribution of small (<5.0 mm) capelin larvae and the distribution of zooplankton smaller than 2 mm at temporal scales equal to or smaller than the tidal cycle. The comparison of short-term vertical displacements of this species with vertical migrations of microzooplankton revealed no relationship that could be directly related to a feeding behavior (Fortier and Leggett 1983).

Capelin are known to ingest particles during the yolk sac stage (Fridgeirsson 1976) but the feeding rates reported are low (Bjørke 1976). In the St. Lawrence estuary, the incidence of feeding in yolk sac capelin is also low and tintinnids are virtually the only prey taken (R. Courtois and J. J. Dodson, Département de biologie, Université Laval, Cité Universitaire, St-Foy (Qué.), unpubl. data). The majority (66%) of capelin larvae captured in the present study were still in the yolk sac stage, indicating that the population sampled had not, on average, reached the phase of mandatory exogenous feeding when copepod eggs and nauplii become the preferred food items (Bjørke 1976). We conclude that no differential mortality or swimming behavior, which could lead to the shaping of larval capelin distribution onto the distribution of early copepod stages, had yet taken place.

Herring

Fluctuations in the abundance of herring larvae and microzooplankton were significantly correlated in the 10- to 20-m and 20- to 40-m depth-strata that comprised 75% of the water column covered by our sampling. The cause for the regular increase in the correlation with depth, a trend that was also evident in the spectral analysis of the data, is not clear. A possible explanation is that fluctuations in the abundance of polychaete larvae that, even when abundant, are not eaten by herring larvae (Marshall et al. 1937; Bainbridge and Forsyth 1971) and that constituted a considerable fraction of total microzooplankton during our sampling are masking the correlation between larval herring and the early stages of copepods in the 0- to 10-m stratum. With the progressive reduction in the abundance of this taxon as depth increased (Fig. 8), the correlation between larval herring and total microzooplankton abundance increased (Table 3). This explanation is consistent with the observation that herring larvae feed selectively (Blaxter 1965; Checkley 1982).

In the deeper strata (10–40 m) where microzooplankton consisted almost exclusively of early stages of copepods, fluctuations in larval herring and microzooplankton abundance were correlated at tidal and subtidal frequencies. A question that arises is whether the congregation of larval herring and its prey at these different scales resulted from active search for higher food concentrations by the larvae or from differential mortality induced by differential starvation (Hunter 1976).

Herring larvae sampled in this study were shown to follow the semidiurnal vertical displacements of their prey over distances of a few tens of metres (Fortier and Leggett 1983). The coherence, at the time scale of the semidiurnal tide, between fluctuations in the abundance of larval herring and microzooplankton in a given stratum is explained by these vertical co-migrations, the two taxa being concentrated in the same depth-interval on a given phase of the tide.

Rosenthal and Hempel (1970) showed that 10-mm herring larvae could perceive prey at an average maximum distance of 10 mm only. Thus, the assumed orientation of swimming toward the level of maximum prey abundance would probably not be visual. Hunter and Thomas (1974) observed that larval northern anchovy swam faster and followed a less variable course at low prey density, whereas they tended to swim slower and more erratically when feeding in a food concentration. Wyatt (1972) also reported that searching activity of plaice larvae decreased as food concentration increased. This behavior could allow fish larvae to find and remain in local clusters of prey (Wyatt 1972). Such a mechanism would explain the concentration of herring larvae within microscale (metres to tens of metres) aggregations of prey on the vertical axis.

The second maximum in coherence between larval herring and microzooplankton abundance occurred at a frequency corresponding to a period of 3.6 h and cannot be explained by the semidiurnal co-migrations of the two taxa. Internal waves with an average period of 3.6 h could produce the subtidal covariability observed in a given depth-interval, since the two taxa were distributed similarly over the vertical axis. However, internal waves of this period were not detected in the time series of physical properties exhibiting a strong vertical gradient such as temperature (Fig. 4b) or salinity. We believe that the subtidal maximum in coherence reflects the horizontal advection past the station of small-scale spatial congregations of herring larvae and microzooplankton. Since the average current speed in the 20- to 40-m depth-stratum was 2.03 km/h, this period of 3.6 h corresponded to horizontal dimensions of approximately 7 km. If we assume that the congregations of herring larvae and microzooplankton are circular at these smaller scales, then the average distance between the respective centers of two adjacent patches would be approximately 7 km and the average distance from a trough to a crest would be approximately 3.5 km. This conversion from temporal to spatial scales is based on Taylor's "frozen field" assumption, which is not rigorously fulfilled in marine environments (Denman and Platt 1975). The value of 7 km should thus be considered with caution and is only indicative of an order of magnitude.

Blaxter (1965), drawing on information given by Bishai (1960), calculated a swimming potential of 20–30 m/h for herring larvae 6.0–8.0 mm in length. Rosenthal and Hempel (1970) reported that herring larvae 8.0–11.0 mm in length covered distances of 1.0–3.0 m/5 min or 12–36 m/h while feeding in tanks. Assuming a daily feeding period of 13 h (Bjørke 1978) and unidirectional movement, herring larvae of the length reported here (average length of 8.95 mm) could cover a maximum distance of approximately 150–500 m/d. In reality the daily net displacement of the larvae in the horizontal plane is not likely to exceed a few tens of metres, since the larvae will not swim continuously in the same direction. Thus, the swimming potential of herring larvae appears to be insufficient to explain directly the correlation found between their distribution and the distribution of their prey at horizontal scales of several kilometres.

In a layered circulation such as the one prevailing in the St. Lawrence estuary, a predator whose vertical displacements are triggered by vertical gradients in prey abundance will tend to accumulate in the richest horizontal concentrations of food advected by the different layers flowing at different levels. This mechanism, which implies only a limited swimming potential, could contribute to the development of a coherence in the distribution of larval herring and its prey at horizontal scales exceeding the ambit of the species. The retention of larval and postlarval stages within tidally energetic areas is a strategy common to several stocks of herring (Iles and Sinclair 1982). The survival value of this strategy could lie in the increased probability of encounter with clusters of prey provided by local gradients in current velocity.

Differential mortality, resulting from local differences in food availability, offers an alternative explanation for a correlation between larval fish and microzooplankton abundance at horizontal scales exceeding the ambit of the larvae (Hunter 1976). Under the differential starvation hypothesis, the development of such a coherence would begin only after the resorption of the yolk reserves. At the level of the population, the exhaustion of the yolk sac (yolk sac frequency ≃ 50%) occurred when the average length of herring oscillated between 8.0 and 9.0 mm (Fig. 3c and 3d, $t = 60$ h to $t = 85$ h). Similar values of length at yolk sac resorption have been found for larval herring of the St. Lawrence estuary (M. Gagnon and H. Powles, Department of Fisheries and Oceans, 15500 Gare Marie Champlain, Quebec, Que. G1K 7K7, unpubl. data). Thus, the first development of a significant coherence in the distribution of larval herring and its food, which occurred at a larval length of 8.0–9.0 mm (Fig. 11), coincided with or came soon after the depletion of the vitelline reserves.

Estimates of growth rate for herring larvae of the St. Lawrence estuary range from 0.15 to 0.27 mm per day (Able 1978; Fortier and Leggett 1982). The transition from an average length of 8.0 mm, prior to which no significant coherence was found between larval herring and microzooplankton abundance, to an average length of 9.0 mm, after which such a coherence appeared fully developed (Fig. 11), represents an interval of approximately 4–7 d (9.0–8.0 mm/0.27 mm per day to 9.0–8.0 mm/0.15 mm per day). These values may represent underestimations, since the growth rate of herring larvae tends to decrease at yolk resorption (Bjørke 1978). If starvation actually controlled the local abundance of herring larvae, then our results suggest that its effect on the distribution of this species became detectable within 4–7 d after yolk sac resorption (Fig. 11). Starving postlarvae would thus appear to vanish faster in the natural environment than in laboratory experiments in which herring larvae survived yolk sac resorption for 12–14 d (Blaxter and Hempel 1963). An increased susceptibility to predation could explain the swift removal of weakened starving larvae in the sea (e.g. Wyatt 1972; Jones 1973; Hunter 1976; Frank and Leggett 1982b).

Dragesund and Nakken (1971) reported a 94% mortality at yolk sac resorption in a concentration of herring larvae sampled off the coast of Norway. This observation and the coincidence in our results between the exhaustion of the vitelline reserves and the development of a correspondence in the distribution of larval herring and microzooplankton suggest (but do not prove) that starvation could influence the local abundance of the early postlarval stages of this species. The actual contribution of differential starvation to the formation of a correlation in the mesoscale distribution of fish larvae and their prey could only be isolated from the effects of kinetic mechanisms by comparing the distribution of a reliable index of starvation (e.g. O'Connell 1980) with the distribution of microzooplankton.

The ecological interpretation of the relationship between first feeding fish larvae and their prey will vary with the scale considered. A simple correlation approach is obviously not sufficient to isolate these dimensions at which a significant link exists. Spectral analysis, which has been successfully used in the study of other trophic levels of the planktonic ecosystem (e.g. Denman and Platt 1975; Mackas and Boyd 1979), allowed us to identify distinct frequency bands or scales at which larval fish and microzooplankton distribution corresponded. The abundance of capelin larvae that were still supported by yolk reserves was not directly related to the abundance of microzooplankton except for large-scale trends. Following the exhaustion of the vitelline reserves, significant covariability developed in the distribution of larval herring and microzooplankton at tidal and subtidal frequencies.

The potential occurrence of significant correspondence at horizontal scales smaller than those resolved by our sampling cannot be discarded (e.g. Sharp 1980; Saville and Schnack 1981). A more complete picture of the coherence spectrum between larval fish and microzooplankton abundance attends the development of higher frequency sampling techniques. A point of the utmost interest for the understanding and prediction of larval fish survival is the fate of these small- and meso-scale congregations of the larvae and their prey following environmental events such as storms and abnormal runoff (Lasker 1975).

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A Gastric Evacuation Model for Young Yellow Perch, *Perca flavescens*

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Gastric evacuation times for age 0+ yellow perch (*Perca flavescens*) (20–69 mm total length) fed continuous meals of zooplankton were measured in the laboratory. The pattern of gastric emptying was described using a segmented regression with two linear sections joined at a fish length of 36.5 mm. It was assumed that evacuation time (hours) is dependent on the amount of food in the stomach; the variables prey size and meal size were the primary determinants of stomach food volume. A gastric evacuation time model was developed; model parameters of evacuation time were quantified as functions of fish size, prey size, and numbers of prey in the stomach. For fish <36.5 mm, all three variables improved the regression model ($P < 0.05$), while for larger fish, only the number of prey in the stomach and fish length contributed ($P < 0.05$) to the explained variance in evacuation time. Estimates of evacuation time were applied to a model of prey consumption and it was found that predicted food intake did not differ significantly from direct counts ($P > 0.05$).

Nous avons mesuré en laboratoire le temps d'évacuation du bol alimentaire de perchaudes (*Perca flavescens*) d'âge 0+ (20 à 69 mm de longueur totale) auxquelles on avait offert de façon continue des repas de zooplancton. Les modalités de l'évacuation gastrique sont décrites à l'aide d'une régression segmentée comprenant deux sections linéaires se joignant à une longueur de poisson de 36,5 mm. Nous supposons que le temps d'évacuation (heures) dépend de la quantité de nourriture dans l'estomac ; les principaux facteurs déterminant le volume de cette nourriture sont les variables taille des proies et volume des repas. Nous développons un modèle du temps d'évacuation gastrique ; les paramètres du modèle sont quantifiés comme fonctions de la taille du poisson, de celle des proies et du nombre de proies dans l'estomac. Chez des poissons plus petits que 36,5 mm, toutes les trois variables améliorent le modèle de régression ($P < 0,05$), alors que chez les poissons plus gros, seuls le nombre des proies dans l'estomac et la longueur du poisson contribuent ($P < 0,05$) à la variance du temps d'évacuation gastrique. Des estimations de ce temps sont appliquées à un modèle de consommation des proies et on constate que l'ingestion de nourriture prédite ne diffère pas de façon significative des comptages directs ($P > 0,05$).

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Studies to quantify food consumption rates of adult fish have received considerable attention (Windell 1966; Magnuson 1969; Elliot 1972; Thorpe 1977; Persson 1979, 1982; Jobling 1981) and generally have demonstrated that weight of food in the stomach declines curvilinearly with time. Consequently, models to predict adult food consumption have assumed the rate of gastric evacuation to be exponential (Eggers 1977; Thorpe 1977; Elliot and Persson 1978). These models are not applicable to young planktivorous fish because gastric digestion is incomplete and the passage rate of prey usually declines with increasing fish size (Laurence 1971; Noble 1973; Mills and Forney 1981). Although there is evidence that initial meals are evacuated more rapidly when followed by additional food and that evacuation time tends to increase with stomach fullness (Noble 1973), the effect of other variables on evacuation rates of young fish has not been adequately evaluated.

Our objective was to examine the influence of prey size, stomach food volume, and fish size on evacuation time and to develop a gastric evacuation time model to estimate food intake by small planktivorous fish. Research focused on age 0+ yellow perch (*Perca flavescens*) of 20–69 mm total length (TL) during a period when they fed on zooplankton.

Methods

Experimental Procedure

Young yellow perch were seined from nearshore waters of Oneida Lake, New York, and held overnight in an 800-L tank containing aerated lake water. Fish were conditioned on experimental prey for 16–18 h and allowed to feed on similar prey during each trial. Prey consisted of *Daphnia galeata* from Oneida Lake and *Daphnia pulex* and *Diaptomus sicilis* from nearby Cazenovia and Fayetteville–Green lakes, respectively. Zooplankton were collected weekly from oblique tows using 153-μm mesh nets; daphnid collections were sieved to retain the larger *Daphnia*, and both *Daphnia* and *Diaptomus* were maintained in laboratory aquaria on blended spinach strained through a 100-μm mesh net.

All trials were conducted in a 15-L continuous-flow cylindrical chamber. The chamber was immersed in an 800-L fiberglass tank filled with filtered Oneida Lake water that served both as a temperature bath and water supply for the feeding chamber. Water temperatures for individual experiments mirrored lake water temperatures. Out of 29 experiments, 4 were conducted at 14 and 15°C and all others were run between 17 and 21°C.

Prior to each trial, 10–20 yellow perch were introduced into the chamber and allowed to acclimate for 1–2 h. Alternate meals of either stained and unstained *Daphnia* or stained and unstained *Diaptomus* were subsequently introduced into the chamber at 20-min intervals for 4 h. *Daphnia* were stained with Bismarck Brown Y, while *Diaptomus*, which were red, were stained with Fast Blue. Groups of fish were allowed to feed on zooplankton for 15 min during which water from the tank was siphoned through the chamber and the flow regulated to $40\ \mathrm{mL \cdot min^{-1}}$ by a thumbscrew clamp. Immediately afterward, the water flow was increased for 5 min to flush uneaten prey from the unit. Fish behaved normally during each flushing and continued to school and feed throughout the duration of each trial. At the termination of each trial all fish were anesthetized with MS-222 (ethyl *m*-aminobenzoate methanesulfonate) and preserved in formaldehyde.

Twenty-nine trials were conducted between 16 June and 20 August 1982 to examine the effects of prey size, meal size, and fish size on evacuation time. Fish 24 to 69 mm TL were fed *Daphnia* that averaged 1.65 ± 0.27 mm (mean ± SD) and *Diaptomus* that averaged 0.90 ± 0.11 mm (mean ± SD) at rates of 40, 80, 120, and 150 $\mathrm{prey \cdot fish^{-1}}$. Mean size of prey fed to fish was determined from length–frequency distributions constructed from 100 or more individuals measured from the anterior margin of the head to the base of either the tail spine of cladocerans or the caudal rami of copepods.

Independent trials correlated the number of bands with the number of times meals of stained and unstained prey were provided. For example, we fed nine yellow perch (33–37 mm TL) stained and unstained meals of 80 *Diaptomus*$\cdot \mathrm{fish^{-1}}$ as described earlier for 2 h. Each band represented 40 min for a total of three bands. Seven of nine fish had three bands each, while two fish apparently did not eat and their stomachs were empty. Additional trials assessed whether food type confounded the effects of prey size on evacuation time. Here, we ran seven trials and provided fish (29–52 mm TL) *Daphnia* or *Diaptomus* meals of 80 $\mathrm{prey \cdot fish^{-1}}$. Both *Daphnia* and *Diaptomus* averaged 0.90 ± 0.11 mm and water temperatures for all trials ranged between 18 and 20°C. Comparison of evacuation times for fish <36.5 mm ($t = 0.91$; df = 24) and ≥36.5 mm ($t = 0.98$; df = 22) fed *Daphnia* or *Diaptomus* of similar size indicated no significant difference ($P < 0.05$) and therefore suggested that *Daphnia* and *Diaptomus* were useful for evaluating the effects of prey size on evacuation time.

Evacuation Time

Evacuation time was defined as the elapsed time between ingestion and evacuation of prey from the stomach and was determined from the number of bands of food in the stomach. A band consisted of a meal of stained and a meal of unstained prey and thus represented 40 min. Fractions of a meal of stained or unstained prey remaining in the stomach were recorded as 0.5 band and represented 20 min. Any further refinement of partial bands was not possible, as demarcation of meals of stained and unstained prey in the intestine was generally undetectable. In all trials, evacuation time (ET) in hours was calculated from the formula

$$\mathrm{ET} = B \times T/60$$

where B is the number of bands in the stomach and T is the time represented by one band or 40 min. Fish with no definite banding were excluded from the analysis. Except for the first feeding trial, which included fish 20–24 mm TL, the number and mean size of prey items in the stomach were recorded and converted to dry weight (milligrams) using length–weight regressions. The effects of prey size, fish size, and stomach food volume on ET were examined by multiple regression analysis (Belanger and Boyle 1980), and an evacuation time model was developed. Stomach food volume was not measured directly but was considered as either the number or dry weight of prey in the stomach. From our estimates of ET, we then estimated the rate of evacuation considered as the ratio of stomach food volume to ET.

Evaluation of Evacuation Time Model

Estimates of food consumption from the evacuation time model were compared with direct measurements of prey consumption. Five trials were run with groups of 15 fish at temperatures between 19.5 and 20.5°C. Fish ranged from 41.1 to 43.6 mm TL and experiments usually ran for 9 h. Before each experiment, fish seined from Oneida Lake were held for 12–14 h in 60-L aquaria containing filtered lake water. Because initial meals may be evacuated more rapidly than subsequent meals (Noble 1973), young perch were fed *Daphnia* stained with Bismarck Brown Y 1 h prior to an experiment and allowed to feed for 0.5 h. Fish were then transferred to 60-L aquaria containing filtered aerated lake water and fed meals of precounted undyed *Daphnia* (<1.5 mm in length) at a rate of $40 \cdot \mathrm{fish \cdot h^{-1}}$. During the course of an experiment, two fish were killed at each of five randomly selected feeding times and the remaining five fish were killed at the termination of a trial for a total of six feeding periods. To minimize regurgitation, young perch were anesthetized in MS-222 and preserved in a 4% formalin–sugar solution. Total lengths (millimetres) were taken on all fish and the number of *Daphnia* in the stomach was recorded. After each experiment, the aquarium was drained and uneaten *Daphnia* were counted. Seven handling experiments were run without fish to determine the loss associated with counting and transfer of prey items. These trials indicated that 96.5% of the *Daphnia* introduced were recovered. Therefore, total consumption corrected for handling losses was estimated from

(No. of *Daphnia* added)(0.965) − No. of *Daphnia* remaining.

Using these five feeding trials, we estimated ET from our multifactor model and the fish size–evacuation time model described by Noble (1973). The amount of food consumed in each time interval was then predicted from the model (Jobling 1981)

$$C_t = S_t - S_0 + A$$

where C_t is the number of prey consumed in t hours, S_t is the number of prey in the stomach at the end of the time interval, S_0 is the number of prey in the stomach at the beginning of the time interval, and A is the number of prey evacuated during the time period estimated as the product of evacuation rate and the period

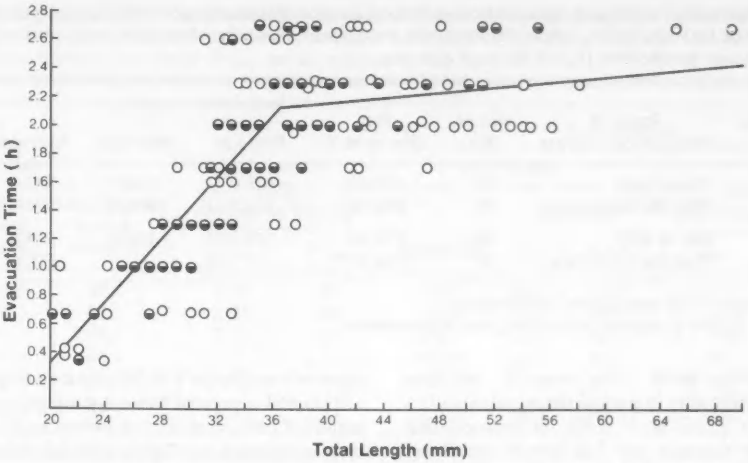

FIG. 1. Evacuation times for young yellow perch 20–69 mm TL fed continuous meals of *Daphnia pulex*, *Daphnia galeata*, or *Diaptomus sicilis* (◓ denotes more than one observation).

of feeding (hours). Total prey consumed over the experimental period was estimated as the product of the number of prey consumed (C_t) by sacrificed fish and the number of fish eating in a given period summed across all six feeding periods.

Results

Gastric Evacuation

ET's estimated for 205 young yellow perch increased linearly with fish length up to about 36 mm and remained rather constant for larger fish (Fig. 1). A two-phase or segmented regression model (Goodnight 1979; Draper and Smith 1981) was used to describe the relation between gastric evacuation and fish size. Using this model, gastric evacuation time (ET) in hours could be predicted from a nonlinear regression containing two linear sections joined at a fish length of 36.5 mm. Accordingly, ET was predicted as

$$\mathrm{ET} = -1.89 + 0.111\ (\mathrm{TL\ in\ mm})$$

for fish <36.5 mm and

$$\mathrm{ET} = 1.87 + 0.008\ (\mathrm{TL\ in\ mm})$$

for fish ≥36.5 mm. The ratio of the regression sum of squares to the total sum of squares for this segmented regression was 0.97. Predicted ET's of 1.2 h for 28-mm fish and 1.9 h for 34-mm fish compare favorably with regression estimates on similar sized fish made by Mills and Forney (1981). Few estimates have been made on larger fish but Noble (1973) did report an ET of 3.3 h for 45-mm yellow perch, which was longer than we observed.

Modifiers of Stomach Food Volume

Noble (1973) demonstrated that ET for young yellow perch increased as the digestive tract filled and suggested a dependence of ET on the amount of food in the stomach. We therefore assumed ET to be dependent on stomach food volume and used the variables of number and dry weight of prey as indices of stomach food volume. Young yellow perch eat increasingly larger prey as fish size increases (Hansen and Wahl 1981), and consequently, we considered the factors of prey size, meal size of large and small prey, and fish size to modify stomach food volume. Fish were sorted into ≥36.5 and <36.5 mm TL groups, and the interaction of these factors on number and weight of prey in the stomach was examined using a stepwise multiple regression (Table 1). In both fish size groups, 49% of the variance in the number of prey in the stomach could be attributed to changes in meal size and prey size. Coefficients were positive for meal size and negative for prey size, indicating that stomach food volume increased proportionately with number fed and was inversely related to prey size. However, fish length did not contribute to the regression at $P = 0.05$. The independent variables meal size and prey size were also identified as modifiers of prey dry weight (milligrams) but only accounted for 13 and 31% of the total variance for large and small fish, respectively.

Evacuation Time Model

In the development of an evacuation time model, the variables of fish size, stomach food volume, and prey size were included because ET changes with fish size (Fig. 1), ET is dependent on stomach food volume (Jobling 1981), and the passage of larger prey through a small tubular stomach may be hindered by physical limitations. To predict ET for age 0+ yellow perch, we used a multiple regression model and included these variables in the form

$$\mathrm{ET} = a + b(X_1) + c(X_2) + d(X_3)$$

where a, b, c, and d are constants and X_1, X_2, and X_3 are stomach food volume measured in number or weight of prey, fish total length (millimetres), and prey size (millimetres), respectively. Regressions were determined for fish sorted into ≥36.5 and <36.5 mm TL and included only those variables that contributed significantly ($P < 0.05$) to a reduction in unexplained variance in ET (Table 2). For both fish size groups, the use of number of prey as an index of stomach food volume resulted in a model that accounted for a higher percentage of the variance in ET than the model incorporating prey dry weight.

TABLE 1. Summary of coefficients obtained from stepwise regression of stomach food volume against prey size, meal size, and fish size for young yellow perch <36.5 and ⩾36.5 mm. Order of addition of variables in stepwise regression is shown. Nonsignificant variables ($P > 0.05$) were excluded.

Fish size group (mm)	Index of stomach food volume	No. of fish	Prey (No. or wt.)[a]	Independent variables[b]		Intercept	R^2
				Prey size	Meal size		
<36.5	No. of prey	95	107(13)	−195.6(1)	1.6(2)	242.6	0.49
	Prey dry weight (mg)	95	896(78)	−536.5(1)	10.8(2)	791.5	0.31
⩾36.5	No. of prey	90	136(14)	−185.9(1)	1.6(2)	254.9	0.49
	Prey dry weight (mg)	90	1106(107)	12.7(1)	—	205.4	0.13

[a] Standard error of the mean given in parentheses.
[b] Order of addition in stepwise regression given in parentheses.

When considering yellow perch <36.5 mm TL, all three variables examined significantly improved the regression ($P < 0.05$) ($F = 70.3$; df = 3, 92; SE = 0.30). In contrast, only number of prey in the stomach and fish length contributed significantly ($P < 0.05$) to the explained variance in ET for fish ⩾36.5 mm ($F = 8.9$; df = 2, 88; SE = 0.29). Although the percentage of the variance in ET explained by the regression (R^2) was higher for small fish than for large fish (70 vs. 26%), the standard errors of the estimate of ET were similar (0.30 for small fish vs. 0.29 for large fish).

Rate of Food Evacuation and Food Consumption

Using estimates of ET derived from our multifactor models for small and large fish, the rate of food evacuation (ER) could be calculated from the ratio of the number of prey in the stomach to ET. For example, a 40-mm yellow perch with 50 prey averaging 0.9 mm in its stomach would have a predicted ET of 2.07 h and an ER of 24 prey·h^{-1}. In contrast, a 30-mm perch with 50 prey of the same size would have an ET of 1.13 h and an ER of 44 prey·h^{-1}. It follows that for different sized fish eating similar numbers of prey, evacuation rate was inversely related to fish size.

Noble (1973) presented evidence that ET could be predicted from fish length alone. We applied estimates of ET using our approach and Noble's to the prey consumption model of Jobling (1981) and compared these consumption estimates with direct counts of prey eaten for fish averaging 42.4 mm (Table 3). The results indicated that Noble's model and our multifactor model underestimated prey consumption by 9.8 and 7.0%, respectively, but were not significantly different from direct counts ($P > 0.05$, paired t-test). Our evacuation time model, however, predicted shorter ET's for all experimental trials than did Noble's and produced significantly higher consumption estimates ($P < 0.01$, paired t-test).

TABLE 2. Coefficients to predict evacuation times of young yellow perch (<36.5 and ⩾36.5 mm TL) from the number of prey in the stomach, dry weight of prey in the stomach (mg), fish length (mm), and average prey size (mm) using stepwise multiple regressions. Nonsignificant variables were excluded ($P > 0.05$, $F < 3.96$)

Fish size group (mm)	Independent variables	Evacuation time equations	
		No.	Weight
<36.5	Stomach food volume	0.00205	0.0978
	Fish total length	0.0995	0.000286
	Prey size	0.367	—
	Intercept	−2.29	−1.79
	R^2	0.699	0.693
	SE	0.300	0.301
⩾36.5	Stomach food volume	0.00118	0.000083
	Fish total length	0.0142	0.012
	Prey size	—	−0.244
	Intercept	1.44	1.94
	R^2	0.262	0.204
	SE	0.293	0.307

Discussion

Laboratory studies to estimate ET were designed to simulate natural conditions by feeding groups of young fish continuous rations of zooplankton. While our results and previous studies (Noble 1973; Mills and Forney 1981) agree that fish size is a primary determinant of ET, the pattern of evacuation has not been firmly established over a wide size range for young fish. Our findings suggest that for continuously fed yellow perch between 20 and 69 mm TL, ET increases linearly with fish size up to nearly 36 mm and then remains rather constant for larger fish. Why smaller fish evacuated prey faster than larger fish may be explained in part by peristalic activity of the stomach induced by distention. Fish <36.5 mm with small stomach capacity averaged only 12% fewer prey by weight (Table 1) than larger fish, and it is likely that their stomachs were more distended than fish ⩾36.5 mm. Under conditions of gastric distention, peristalic activity induces secretion of gastric enzymes and acid (Norris et al. 1973) and probably increases muscular action and enzyme activity to increase rates of digestion and evacuation (Jobling 1981). Interpretation of the ET–fish size results can also be facilitated from knowledge of stomach maturation. In over 100 digestive tracts of fish <20 mm, the stomach and intestine were indistinct. As young perch reached 30–40 mm, the stomach became a distinct sac and the pyloric region was nearly fully developed, and for larger fish, stomach capacity tended to increase with fish size. It follows that increased storage capacity will prolong food passage and that small fish with a digestive tract resembling a simple straight tube would possibly have less control over prey passage and therefore evacuate prey faster than large fish with a distinct sac-like stomach and intestine.

Implicit in gastric evacuation models for adult fish is the assumption that volume of food in the stomach will influence gastric ET (Jobling 1981). Hence, it was reasonable to assume that stomach food volume could also influence ET in young fish. To evaluate this premise, we examined several factors and

TABLE 3. Comparison of observed and predicted estimates of food consumption of age 0+ yellow perch collected 7 August 1981. Prey consumption was estimated according to Jobling (1981) and evacuation times were estimated using our multifactor evacuation time model (ET_M) and the evacuation time model of Noble (1973) (ET_N). The abbreviations C_N and C_M were used to denote consumption estimated according to Noble and our multifactor model, respectively.

Fish			Mean evacuation time (h)		Consumption				
					No. of prey			Deviation from observed (% ±)	
						Predicted			
No.	Mean size (mm)	Mean No. of prey in stomach	ET_N	ET_M	Observed[a]	C_N	C_M	C_N	C_M
15	43.6	50.9	2.22	2.12	2461	2428	2502	−1.3	1.6
15	42.1	54.2	2.21	2.09	2284	2216	2298	−3.0	0.6
15	41.1	63.0	2.20	2.10	3081	3352	3453	8.8	12.1
15	42.8	61.1	2.21	2.11	4326	3500	3609	−19.1	−16.1
15	42.2	62.1	2.21	2.11	4212	3262	3352	−22.6	−20.4
Mean	42.4	58.3	2.21	2.11	3273	2952	3043	−9.8	−7.0

[a] Corrected for losses due to transfer, counting, and recovery.

found that meal size and prey size were primary determinants of stomach food volume as measured in number or weight of prey for young yellow perch. Since growth of young perch in the wild is dependent on prey abundance (Mills and Forney 1981), we must infer that these fish eat more at higher prey densities. Consistent with this pattern, our experimental results indicated that the number of prey in stomachs of perch ≥36.5 and <36.5 mm was dependent on meal size. It follows that if the evacuation rate (number of prey egested per hour) increases with more food in the stomach (Noble 1973; Jobling 1981) then the digestion rate should increase with more available prey. This pattern is analogous to that found by Werner and Blaxter (1980); they showed that the rate of food passage for larval herring (*Clupea harengus*) increased at higher prey densities. We might also anticipate that prey size would influence stomach food volume, since juvenile fish generally prefer small over large prey items (Feller and Kaczynski 1975; Furnass 1979; Hansen and Wahl 1981). It follows from our regression model that stomach food volume was dependent on prey size, and numbers of prey in stomachs of young perch were inversely related to prey size.

While we found that prey size and meal size accounted for 49% of the variance in number of prey in stomachs of young yellow perch, other factors can modify feeding behavior. Even though laboratory light conditions were similar for all our experimental trials, yellow perch fry are visual feeders and therefore changes in light intensity can alter visual acuity (Vinyard and O'Brien 1976; Confer et al. 1978). A reduction in visual acuity reduces the effective density of zooplankton, which in turn may adjust the feeding rate and prey selectivity (Werner and Hall 1974). Feeding history and experience also influence predation, as illustrated for rainbow trout (*Salmo gairdneri*) by Ware (1971). While we did acclimate our fish to experimental prey, feeding history in the lake could have influenced laboratory feeding behavior. Stomach contents in fish collected from the lake population commonly had prey items that were unlike those used in feeding trials. However, recent results for 25-mm walleyes (*Stizostedion vitreum vitreum*) conditioned only to crustacean zooplankters indicate that these fish could switch very rapidly and completely to different prey when available (Mathias and Li 1982).

Noble (1973) found that ET varied directly with size of young yellow perch. Our results agree with Noble's and also indicate that ET is dependent on the volume of food in the stomach and prey size. For fish <36.5 mm, stomach food volume, fish size, and prey size all contributed significantly to the regression ($P < 0.05$) and accounted for 70% of the variance in ET. For larger fish, ET was longer and both stomach food volume and fish size contributed significantly to the regression model. It is interesting that the factor of prey size influenced ET's of small fish (<36.5 mm) but did not for larger fish. Whether this effect on ET for small fish was in response to changes in prey species or prey size remains unclear. Differences in digestibility (Fange and Grove 1979) between food items would favor a prey species effect, but this explanation was negated by evidence that large and small fish did not respond similarly to contrastingly sized prey species. For small fish, we can only speculate that large prey are retained longer due to physical limitations of the passage of a large particle through a small tube. It follows that for larger fish with larger stomach capacity, 0.9-mm *Diaptomus* and 1.7-mm *Daphnia* would both be small relative to stomach size, and we might, therefore, expect prey size to have little or no effect on ET's of these fish. Finally, we recognize that temperature affects evacuation rates for several fish species (Elliot 1972; Persson 1982), but we did not include it in our model, since most of our experiments were conducted between 17 and 21°C; Mills and Forney (1981) found no significant difference in gastric ET for young yellow perch within this temperature range.

The Noble evacuation time model assumed that ET was constant regardless of the amount or type of food in the stomach. Consequently, his model would tend to overestimate ET and thereby underestimate prey consumption in at least two field conditions where (1) fish have few ingested prey because there are few prey in the environment and (2) fish have depleted the preferred prey size and have been forced to eat greater numbers of very small prey. In contrast, our evacuation time model does not assume a constant ET for a given sized fish, since ET will change with the quantity of prey in the stomach and prey size. For example, if a 35-mm yellow perch had 10 prey averaging 1.0 mm in its stomach, our evacuation time model would predict an ET of 1.58 h. Further, if the same sized fish had 20 times as many or 200 prey of only 0.5 mm in its stomach, ET would be 1.78 h. The Noble model would predict ET to be 1.99 h for both situations. When we convert these ET's to the number of prey egested per hour, Noble's estimates of ET would result in consumption rates 10–21% lower than would be calculated using our multifactor model. These hypothetical results are further confirmed by laboratory evidence (Table 3) indicating that ET's for yellow perch of 42.4 mm mean size and fed at a low feeding rate (40 prey·fish^{-1}·h^{-1}) were significantly longer using the Noble model compared with ours.

How closely our multifactor model approximates ET for age 0+ yellow perch in the lake is uncertain, but we do know that it reflects the broad range in stomach food volume (Noble 1975; Mills and Forney 1981) one might observe and the size-selective feeding behavior (Hansen and Wahl 1981) of young fish. While we did not detect a significant difference ($P > 0.05$) between observed and predicted estimates of young yellow perch consumption, our evacuation time model may underestimate consumption for a couple of reasons. First, an overestimate in the number of bands could result in an underestimate in prey consumption. We recorded partial meals of stained and unstained prey remaining in the stomach as 0.5 band. Consequently, 1.5 band could represent an ET of 60 or 41 min, or anywhere in between, thereby leading to a possible underestimate in prey consumption. Second, gastric ET is sensitive to variation in feeding regime and ET usually increases as the digestive tract fills (Noble 1973). While confined fish were conditioned overnight on experimental prey before each feeding trial, we recognize that these same fish were also starved 1–2 h before each experiment. We can only speculate that digestive tracts of confined fish were not full at the beginning of each trial, and therefore our evacuation model would predict slower rather than faster evacuation of initial meals.

Finally, we present evidence that ET's of young yellow perch are determinable from the factors of fish size, prey size, and the number of prey in the stomach. It follows that variation in the shape of the diel feeding curve will influence ET; we feel that the best estimates of daily ration can be obtained through sampling at frequent intervals during the day using our evacuation time model. While our model assumes continuous feeding, it is likely that laboratory yellow perch seldom fed continuously judging from the variability in stomach food volume and the span of ET's for similar sized fish (Fig. 1). Such among-fish variability in stomach prey volume is consistent with field observations (Mills and Forney 1981) and suggests that these fish may also feed discontinuously in the wild. To compensate for such among-fish variability, an increase in fish gut sample size would be required. Recognizing such limitations, we believe our multifactor evacuation time model is a refinement of earlier models and represents a necessary step in the development of a general model of predation by young planktivorous fish.

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NOTES

Dual Cannulation of Free-Swimming Atlantic Salmon, *Salmo salar*

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Nichols, D. J., and M. Weisbart. 1984. Dual cannulation of free-swimming Atlantic salmon, *Salmo salar*. Can. J. Fish. Aquat. Sci. 41: 519–521.

We describe a technique for enduring (>1 mo) dual cannulation of large, free-swimming Atlantic salmon, *Salmo salar*. Repeated blood sampling is possible without disturbance of the fish and the technique is suitable for studies that involve administration of radiolabeled substances for kinetic and distribution volume measurements.

Nous décrivons dans l'article qui suit une méthode d'insertion permanente (de plus d'un mois) d'une canule double chez des saumons atlantiques, *Salmo salar*, de grande taille et nageant librement. Il est possible ainsi de prélever des échantillons répétés de sang, sans déranger le poisson. En outre, la méthode convient à des études impliquant l'administration de substances marqués aux isotopes dans le but de faire des mesures de cynétique et de distribution de volumes.

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There is increasing interest in kinetic and distribution volume measurements in fish (e.g. Eales et al. 1982). These measurements require injection or infusion of radioisotopically labeled compounds (or dye compounds) and sequential blood sampling thereafter. Techniques currently in use involve single dorsal aortic catheters through which compounds are administered and samples are drawn (e.g. Smith and Bell 1964). Adsorption of infused tracer to the inner wall of such can compromise blood samples subsequently drawn from the catheter (Fletcher et al. 1969). An alternative method to avoid contamination of the blood sample is to inject labeled compound directly into the fish (via the heart or caudal artery) and subsequently remove blood samples from the catheter. This, however, involves anesthetizing and handling the fish: procedures that can cause prolonged disturbances in many physiological processes (e.g. Houston et al. 1971).

Soivio et al. (1975) reported a stabilization of hematological measurements when rainbow trout (*Salmo gairdneri*) were restricted in a cylinder compared with free-swimming fish. However, moderate confinement of juvenile chinook salmon (*Oncorhynchus tshawytscha*) caused an increase in plasma cortisol levels (frequently used as an indicator of "stress," Donaldson 1981) compared with unconfined fish (Strange et al. 1978) and may represent a more "unnatural" situation in salmonids than in free-swimming fish. Our objectives were to insert two cannulae in large Atlantic salmon (*Salmo salar*) without major surgery, to maintain free-swimming fish for experimentation, and to take blood samples and inject or infuse labeled compounds without disturbance of the fish. Several dual cannulation techniques have been reported for teleosts, most of which involve cannulation of the dorsal and ventral aorta (e.g. Saunders and Sutterlin 1971; Garey 1969; Jones et al. 1974). However, none of the techniques satisfy our objectives due to short durability of the preparation, restraint of the fish, or disturbance during sampling.

Methods

Healthy Atlantic salmon, 2–9 mo after spawning, were held in freshwater at 10°C for at least 2 wk prior to surgery. Fish were anesthetized with 0.04% 2-phenoxyethanol. Two cannulae (PE 50), 1–1.25 m long, were inserted into the dorsal aorta through a size 18 thin-wall needle (Houston 1971) between the first and second gill arches using the general surgical procedures of Smith and Bell (1964). The two cannulae were sutured to the roof of the mouth and to the dorsal edge of the operculum. After surgery, the fish length and weight were recorded (ranges: weight 1–4 kg, length 50–90 cm) and the fish placed either individually in 100-L tanks equipped with power filters but without directional flow or in 600-L Living Streams (Frigid Units Inc., Ohio) with directional flow. Dechlorinated freshwater was dripped into the tanks at an exchange rate of approximately 10%/d. The tanks were completely screened but subject to a natural photoperiod from fluorescent lights that simulated natural photoperiod; the Living Streams were equipped with a small viewing port through a side window of the tank.

Before the fish fully recovered from surgery, the two cannulae were tied together at 8-cm intervals with silk thread, and 8-cm lengths of plastic straw were placed over the entire length of the cannulae except for 4 cm at the end. The last section of straw was taped to the top edge of the tank with the cannulae protruding beyond the screen. The catheters were filled with saline containing 250 IU ammonium heparin/mL and plugged with stainless steel pins. Attached to one of the pins was a short length of cannula (1 cm) and a second pin bent at right angles to prevent the cannulae slipping into the tank. Blood samples were taken as described by Smith and Bell (1964).

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We infused materials by attaching one end of a cannula (1–1.5 m) to a syringe pump and the other to the cannula. To demonstrate the potential error involved in using only one cannula for infusion of labeled material and blood sampling we infused one salmon with a 0.7% saline solution with 4% ethanol containing approximately 55.5 kBq [^{3}H]cortisol/mL at a rate of 0.6 mL/h. After 12 h the infusion was stopped, the saline in the cannula was removed along with the first 200 μL of blood before we quickly took 0.5-mL blood samples (total sampling time <2 min); the blood was centrifuged and aliquots of the plasma were counted in a liquid scintillation counter.

To determine if blood sampling disturbed the fish we measured plasma cortisol levels using a specific radioimmunoassay (Weisbart and Jenkins 1981) in sequential blood samples (0.3 mL removed) taken at 0, 2, 4, and 9 h. To assess postsurgical recovery we took blood samples from fish between 1 and 5 d postsurgery and from noncannulated fish sampled within 3 min of disturbance for measurement of plasma cortisol.

Results and Discussion

Dual cannulation of large salmon was achieved without major surgery by inserting two small cannulae into the dorsal aorta. Postmortem examination of the fish indicated that the cannulae did not seriously obstruct the blood vessel in the size of fish used. We cannot exclude the possibility that there is altered cardiovascular function. However fish were kept for >1 mo postsurgery, in good condition and with intact, functioning catheters, before the sutures attached to the operculum eroded.

Of 29 fish cannulated, 1 fish failed to recover from surgery and 2 fish lost blood (hematocrit 10.5 and 11.3%). The hematocrit was 26.4 ± 1.7 (mean ± SE, $N = 28$) 1–5 d postsurgery, which is similar to that reported for cannulated salmonids (e.g. Smith 1966; Soivio et al. 1975).

There is potential error of using a single catheter for measuring [^{3}H]cortisol levels in plasma after infusion of [^{3}H]cortisol (Table 1). Even after removal of saline and the first 200 μL of blood, measurement of ^{3}H levels in plasma for the next 2 mL of blood would lead to an overestimation of between 14 and 1800%. Further, since pure [^{3}H]cortisol levels in Atlantic salmon plasma after infusion of [^{3}H]cortisol are less than 30% of the total ^{3}H in plasma, this error will be magnified. Since the degree of binding of radiolabeled substance to a cannula varies, depending on both the substance and vehicle (Fletcher et al. 1969), it is important to determine this error in each situation.

Prior blood sampling does not affect subsequent plasma cortisol levels (Table 2). Plasma cortisol levels are highly variable; this is not due to assay noise (interassay coefficient of variation <10%) but occurs both among and within fish and many represent episodic secretion of cortisol (e.g. Bry 1982). Observation of the fish through a viewing port indicated lack of disturbance of the fish during sampling. The lack of disturbance is probably due to the arrangement of the cannulae such that remote sampling is possible without entanglement of the catheters or obvious distress to the fish. As the fish swam around in the tank the cannulae rotated inside the plastic straws. The straws gave slight buoyancy, provided little frictional resistance to the rotation of the cannulae, and prevented kinks from occurring.

TABLE 1. Levels of radioactivity in blood samples (hematocrit = 28.6%) taken through a cannula from one Atlantic salmon after infusion of 33.3 kBq [^{3}H]cortisol/h through the same cannula.

Volume of blood removed (mL)	^{3}H levels in blood (DPM/0.5 mL)
0.5	83 507
1.0	18 739
1.5	7 521
2.0	4 898
2.5	4 397
3.0	4 432
3.5	4 338
4.0	4 023

TABLE 2. Plasma cortisol levels (mean ± SE, $N = 4$) in Atlantic salmon blood sampled repeatedly over 9 h.

Time (h)	Plasma cortisol[a] (ng/mL)
0	122.6±39
2	59.8±15
4	75.6±15
9	70.1±23

[a] No significant differences over time using the Friedman nonparametric ANOVA ($\chi_r^2(4,4) = 5.7$, $0.1 < P < 0.2$) (Siegel 1956).

TABLE 3. Plasma cortisol levels (mean ± SE (N)) after surgery in Atlantic salmon.

Days postsurgery	Plasma cortisol[a] (ng/mL)
0[b]	52.0±14.7 (3)
1	59.4±6.3 (4)
3	93.7±10.6 (10)
4	78.3±12.7 (8)
5	92 (2)[c]

[a] No significant differences over time using Kruskal–Wallis nonparametric ANOVA ($H = 6.81$, df 3, $0.05 < P < 0.1$) (Siegel 1956).

[b] Fish were noncannulated and sampled via the caudal artery <3 min after disturbance.

[c] Not included in statistical analysis.

Plasma cortisol levels in dual-cannulated salmon were not statistically different 1–5 d postsurgery from noncannulated salmon (Table 3). We suggest that the recovery period after cannulation in salmon is similar to that of rainbow trout (1–3 d, Bry and Zohar 1980). Plasma cortisol levels in adult Atlantic salmon were higher than those reported for rainbow trout

(<20 ng/mL) (Bry and Zohar 1980); cortisol values were also higher in salmon than in noncannulated and (single) cannulated brook trout, *Salvelinus fontinalis* (<20 ng/mL) (D. J. Nichols and M. Weisbart, unpubl. data).

In conclusion, the dual cannulation technique in large free-swimming salmon allows blood sampling without disturbance and maintenance of fish for periods >1 mo. The technique is particularly suitable for kinetic studies on "unstressed" fish, studies that involve injection or infusion of radiolabeled substances.

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Crab Response to Prey Density Can Result in Density-Dependent Mortality of Clams

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Field experiments involving recovery of marked clams showed that even adult native littleneck clams (*Protothaca staminea*) could suffer high mortality to cancrid crabs. The mortality rate increased with clam density, suggesting that these crabs preferentially forage in areas of higher prey density. Acoustic telemetry showed that at least one of the crabs, *Cancer productus*, is sufficiently mobile to search large areas while foraging. This is important for understanding the effects that these large, mobile predators have on the distribution and the abundance of their prey and may explain the high incidence of crab predation seen in mariculture operations where the density of clams is artificially raised.

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Des expériences de marquages–recaptures de clams sur le terrain démontrent que la prédation par les crabes cancrides peut infliger d'importantes mortalités, même chez la clam indigène adulte, *Protothaca staminea*. Le taux de mortalité augmente en fonction de la densité des clams, ce qui donne à penser que ces crabes recherchent leur nourriture dans les zones de forte densité des proies. La télémétrie acoustique indique qu'au moins une espèce de crabes, *Cancer productus*, est suffisamment mobile pour couvrir de grandes superficies quand elle est en quête de nourriture. Ceci est important pour bien comprendre les effets de ces prédateurs mobiles sur la distribution et l'abondance de leurs proies, et peut expliquer la forte incidence de prédation par les crabes observée en mariculture quand la densité des clams est artificiellement accrue.

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Crabs are important predators on intertidal bivalves and in some areas may be responsible for the observed low densities of wild clams and the poor survival of planted hatchery-reared clams (e.g. Whetstone and Eversole 1978). Their high mobility and complex behavior makes it difficult to manipulate their density, and most investigations of their impact have relied on stomach analysis and laboratory (e.g. Walne and Dean 1972) studies rather than field experiments. Field manipulation of clam density is practicable (Peterson 1982a). Therefore, by experimentally manipulating the density of littleneck clams (*Protothaca staminea*), we examined the influence of their relative density on mortality from crabs.

Site and Methods

Field experiment — A study area was selected in Grappler Inlet (48°50′N, 125°6′W) near Bamfield Marine Station. The site was protected from wave exposure. The sediment was pebbles mixed with fine sand with a mean particle size of 6.6 mm ($M_\phi = 2.7$) and poorly sorted ($\sigma_\phi = 1.8$; Inman 1952). Most natural bivalves at a tidal height of 1.0 m were small tellinids although the venerid clam *P. staminea* was present at densities up to 20 clams/0.25 m^2 just above this (Table 1).

Four species of crabs, *Cancer productus*, *C. gracilis*, *C. magister*, and *Pugettia producta*, all capable of preying on large clams (E. G. Boulding, pers. obs.), were caught in side-entry crab traps subtidally below the study area.

On a 24-m transect parallel to the water's edge at a tidal height of 1.0 m, 10 points were selected randomly and each assigned randomly to one of two treatments "sparse" and "dense." Fences of 4-mm VEXAR mesh (0.5 × 0.5 × 0.15 m (high)) were carefully buried at these points, leaving 10 mm protruding above the surface. Native littleneck clams were air-dried, sprayed with orange paint, and divided into three size-classes (30–35, 40–45, and 50–55 mm). Five individuals of each size-class were placed in the sparse treatments and 20 of each were placed in the dense treatments.

Care was taken to disturb as little as possible the sediment and natural infauna being enclosed. This meant that the initial density of natural clams was not known and could only be estimated from that of surrounding areas. Thus for this first run, the sparse treatments contained 15 *Protothaca* plus whatever clams were present naturally, while the dense treatment contained 60 *Protothaca* plus the natural clams. This added to the variance among replicate enclosures, since some of the clams ultimately eaten were the unmarked natural ones, but reduced the probability of experimental artifacts from disturbing the sediment. Temporary covers of 4-mm VEXAR were sewn on the fences for the 1st wk. These were removed December 9, 1981.

The experiment was visited semimonthly and any orange shell fragments visible within the fences were collected. Shell fragments found outside the fences were collected and their positions were recorded. After 6 mo (May 26, 1982) the fences were removed and the surface was combed first with fingers and then with a clam fork, and any live clams or fragments were set aside. All the sediment that had been enclosed was dug to a depth of 0.3 m then sieved through a floating sieve (0.5 × 0.5 m) of 5-mm mesh. The marked clams that had been placed in each enclosure were separated into four categories: alive, crab-crushed, dead undamaged, and missing. The number of clams in the crab-crushed category was determined by counting hinges. In addition, the numbers of natural bivalves of shell length $\geq$10 mm, crabs, and predaceous gastropods present in each enclosure were noted.

In Grappler Inlet, shells of adult clams opened by crabs can be distinguished from those of clams opened by the other predators (starfish, small mammals, birds, predatory gastropods and octopus). Only shells dropped by birds are likely to be confused with those crushed by crabs, but with experience these can be distinguished by appearance and location. In contrast with the relatively straight edges found on pieces of shells dropped by birds (E. G. Boulding, pers. obs.), the method of attack used for clams large relative to the crab often leaves characteristic chips on the ventral margin and a U-shaped channel with ragged edges (Boulding 1983). Shell fragments of the Japanese littleneck clam, *Tapes phillipinarum*, a species generally found living higher in the intertidal and shallower in the sediment than *Protothaca*, were found on the rocky outcroppings bordering the study site, but bird predation on adult *Protothaca* appeared to be very low.

This experiment was repeated in the summer for only 3 mo (June 5, 1982–Sept. 1, 1982). Three changes were made to reduce problems encountered in the first experiment: (1) only the smaller two size-classes of clams were used because clams of the largest size-class appeared unable to rebury themselves successfully, (2) each of the two size-classes used were spray painted with a different color of paint, and (3) each clam had its enclosure number marked on it with permanent felt pen to allow identification of stray clams.

Laboratory experiments — To determine approximate maximum feeding rate of large *C. productus*, four male crabs (carapace widths 111–170 mm) were held in individual containers and starved for 1 wk. They were then offered 12 clams, 4 of each of three size-classes (shell length 31–35, 45–48, and 58–60 mm). The number of clams eaten of each size-class was recorded after 24 h.

To determine whether paint affected the palatability of clams to crabs, five *C. productus* (carapace width 89–111 mm) and

TABLE 1. Natural clam densities (per 0.25 m^2) of clams $\geqslant$10 mm in shell length above and below the site of the field experiment.

Clam species	Above site (1.4 m)[a]			Below site (0.9 m)[a]		
	Mean	SD	Range	Mean	SD	Range
Macoma inquinata	28.7	13.72	10–39	7.75	2.986	5–14
Saxidomus giganteus	24.5	12.12	8–37	1.50	1.000	1–3
Protothaca staminea	13.5	5.507	8–20	0.25	0.500	0–1

[a] Sampled April 1982 ($N = 4$).

TABLE 2. Prey density manipulation experiment. Number of clams recovered in each of four categories: alive (AL), crab-crushed (CC), dead undamaged (DU), and missing (M) for both the winter–spring (WSp) and the summer (Sum) runs. Total number of clams initially planted was 5 of each size-class for the sparse treatments and 20 of each size-class for the dense treatments (see Fig. 1 for means and standard errors and text for analysis).

Treatment	Small clams				Medium clams				Large clams			
	AL	CC	DU	M	AL	CC	DU	M	AL	CC	DU	M
WSp, sparse	21	1	1	2	18	4	1	2	13	11	0	0
WSp, dense	55	32	2	11	60	30	0	10	48	30	3	19
Sum, sparse	19	4	0	2	18	4	0	3	—	—	—	—
Sum, dense	54	42	2	2	54	41	0	4	—	—	—	—

one *C. magister* (154 mm) were offered five painted and five unpainted clams (shell length 30–35 mm). Clams were replaced as they were eaten. All crabs were held in individual containers in free-running seawater.

Crab movement — Two *C. productus* were captured in Grappler Inlet with side-entry crab traps. A transmitter (Smith-Root Electronics model SR69A) was fastened to each crab's carapace. The crabs were released at the point of capture on the day after capture and were located daily from a rowboat using a directional hydrophone (Smith-Root Inc., model SR-70H). The position of the crab and the depth of the water were recorded on a map prepared from aerial photographs. To test for behavioral artifacts caused by the transmitter, another crab was fitted with a "dummy" transmitter and observed in the laboratory.

Results

Field experiment — Although the small and medium clams reburied themselves to normal depths, this was not true of the large clams used only in the first run of the experiment. Some of the large clams did not rebury and as a result began to gape allowing crabs easy access.

The small (30–35 mm) and medium (40–45 mm) clams showed significantly higher mortality rates from crabs in the dense treatments than in the sparse treatments during both the winter–spring run (Fig. 1a; two-way Friedman rank sums test on cell medians, $P < 0.05$) and the summer run (Fig. 1b, $P < 0.05$). There was no significant difference between the two treatments for the large (50–55 mm) clams used only during the winter–spring run. There was also no significant difference in mortality rate between the small and medium size-classes for either run of the experiment. In the winter–spring run, 44 clams were classified as missing (Table 2). Fifteen of these, with shells chipped by crab attack, were found up to 25 m away in the eelgrass below the fences. Clams were also found in the eelgrass in the summer run but because they were marked with their replicate number they could be assigned to a treatment and included in the crab-crushed category. The dead undamaged category had only a total of nine clams in both runs. No clams were found drilled by predatory gastropods.

At sampling, the density of natural (unmarked) bivalves in the enclosures was similar to the density just below the site, but

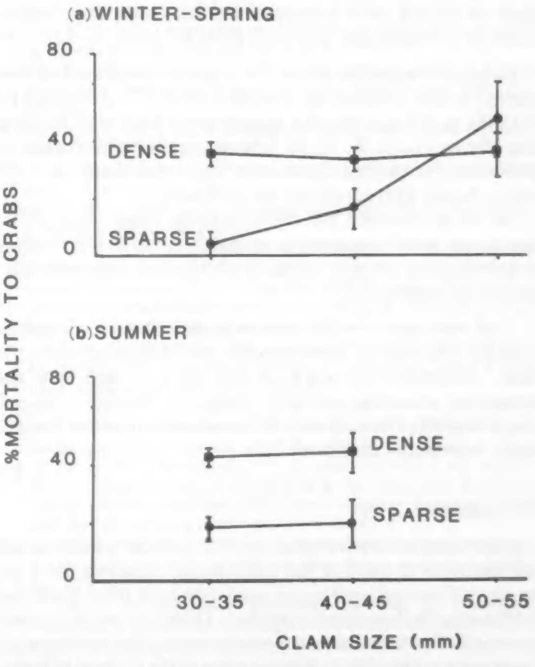

FIG. 1. Mean percentage (with standard errors) of clams classified as eaten by crabs (i.e. number crab-crushed divided by the total number recovered, see Table 2 for summary of number in each treatment and category combination).

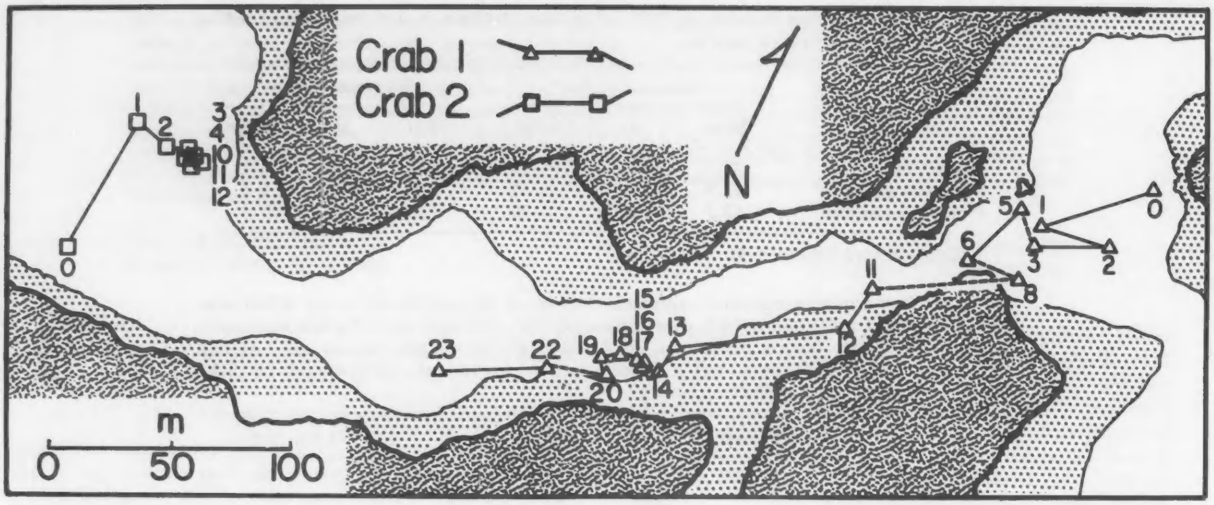

FIG. 2. Net daily movements of the two crabs tracked in Grappler Inlet using acoustic transmitters. The numbers on the points represent the days that have elapsed since the crab was released. The clear areas are subtidal, the stippled areas are intertidal and the mottled areas are land above highwater.

fresh unmarked shell fragments indicated that some natural clams in the enclosures had been eaten by crabs.

Laboratory experiments — The four male crabs that had been starved in the laboratory ate a mean number of 3 clams each (SE = 0.48) in 24 h and showed no preference for any of the three size-classes (Table 3). In the laboratory, the crabs showed no preference for painted clams over unpainted clams (N = 51 clams; G-test with correction for continuity).

We often observed the crabs carrying clams about in the aquariums, which suggested to us that they also do it in the field; a crab carries a clam by using its chelipeds to brace the clam against its mouthparts.

Crab movement — One crab was tracked for 23 d, and the other for 13 d (Fig. 2). Both tended to stay in water at least 1.0 m deep, sometimes spending several days in one spot and sometimes traveling >50 m/d (Fig. 2). Neither crab left Grappler Inlet. The crab with the simulated transmitter ate well under laboratory conditions.

Discussion

Crab response to patch density — In both the winter–spring and the summer runs of the experiment, clams in the high-density patches suffered higher predation rates from crabs than did those in the low-density patches. Three explanations could account for the differences in mortality rates: (1) crowding made clams more vulnerable by forcing them to the surface, (2) areas of high clam density attracted crabs from a distance, and (3) crabs returned to patches where their success had previously been high.

Native littleneck clams forced to live near the surface are more vulnerable to predation by *C. magister* than those allowed to burrow to normal depths (Pearson et al. 1981). However, we doubt that the clams in our dense treatments were crowded enough to cause upward displacement; natural densities in nearby Roquefeuil Bay commonly exceed the highest density used in the experiment without evidence of this. Indeed, field densities elsewhere of large (>20 mm) *P. staminea* have been reported to reach over 70 clams/0.25 m^2 (Paul and Feder 1973).

TABLE 3. Percentage eaten from size-class of clams during 24 h by male *C. productus* starved for the previous week. N = total number of clams eaten by each crab. They were initially offered four clams of each size-class.

Crab carapace width (cm)	Clam shell length (cm)			
	3.1–3.5	4.5–4.8	5.9–6.0	N
11.1	2	0	1	3
13.8	1	0	1	2
14.8	1	2	1	4
17.0	2	1	1	4
Total	6	3	4	13

Crabs may be able to detect areas of higher prey density from a distance and selectively forage there. *Cancer magister* uses its antennules as distance chemoreceptors and has the ability to detect extracts of the native littleneck clam at a concentration of 10^{-10} g/L (Pearson et al. 1979); higher concentrations of clam extract were shown to initiate probing with the chelae and walking legs and to cause other feeding behaviors. If many clams in a given area emit a greater concentration of metabolites than would fewer clams in the same area, more crabs would be attracted, thus causing higher mortality rates. Our observations of crabs congregating in dug-over areas of the beach suggest that they use distance chemoreception to find prey.

Finally, even without distance chemoreception crabs might cause density-dependent mortality by remaining longer in, or returning more often to, patches of high density, as would be predicted by optimal foraging hypotheses (e.g. Charnov 1974). To obtain a clam a crab must first locate the clam's exact position by touching it with mechanoreceptors and/or contact chemoreceptors on its claws and walking legs (Case 1964;

Warner 1977) and then dig it up. The average time required to probe for and to dig up clams would be less in high-density patches.

The acoustic telemetry data indicate that these crabs are sufficiently mobile to search large areas during foraging (Fig. 2). This mobility could enable them to respond to and exploit differences in prey density over considerable distances. Thus, these crabs may spend more time searching for prey in regions of Grappler Inlet where the average prey density is higher. Hill (1979) showed that the mangrove crab (*Scylla serata*) spent more time in the regions of the estuary that had the highest number of prey organisms.

The high mortality of marked littleneck clams probably resulted from the lack of alternative prey in Grappler Inlet, which is probably due in turn to an abundance of predators including the three cancrid crabs: *C. productus*, *C. magister*, and *C. gracilis*. The low densities of bivalves and polychaetes that occur naturally in the soft sediment areas and of small balanoid barnacles and large thick-shelled snails (*Thais lamellosa*) that occur in the rocky areas of Grappler Inlet might represent a lower profitability for crabs than does a high density of large bivalves. When given a choice of littleneck clams, *C. productus* prefers the smallest (Boulding 1983, 1984) but the starved crab experiment suggests that these crabs are less selective when food is scarce. This is also true of the green shore crab (*Carcinus maenas*) preying on mussels (*Mytilus edulis*) (Jubb et al. 1983).

Although this is the first study to show that crabs can cause density-dependent mortality of clams, examination of data from other workers suggests that this phenomenon may be widespread. Peterson (1982a, 1982b), in his studies on the population biology of intertidal clams, has obtained disappearance rates of up to 54% of the clams he originally planted. In a field experiment with *P. staminea* in enclosures, the proportion of missing clams appears to be significantly density dependent 3 out of 12 times. Although Peterson (1982b) attributed the loss of these clams partly to emigration by the clams themselves, he found that the missing proportion was significantly reduced (from 33 to 2%) by the presence of complete cages. Our results suggest that these missing clams were carried off by crabs to be opened in safer surroundings such as subtidal eelgrass beds.

Effect of crabs on their prey populations — Estimation of the extent to which these crabs determine the abundance of their prey is difficult because of their mobility, their broad diet, and the potential complexity of their response to the relative abundances of their various prey. The impact of a highly mobile predator with a broad diet, such as a crab, on a prey population would be expected to be quite different from that of slow-moving predators such as snails because of the difference in the distances over which the predators can respond to differences in the density of their prey. Although the slow moving whelk *Busycon* also uses chemoreception to detect clams and must dig them out of the sediment (Carriker 1951), whelk predation on hard clams was never found to be density dependent (Peterson 1982a). The density-dependent feeding response shown by these crabs probably results in a more uniform distribution of their prey so long as the crabs are reasonably abundant. If a similar response to prey density occurs in other decapods it might explain why American lobsters (*Homarus americanus*) and cancrid crabs prevent outbreaks of sea urchins (e.g. Wharton and Mann 1981) even though sea urchins are not a preferred prey when urchin densities are low (e.g. Breen 1974). It may also explain why mariculture projects that artificially increase the local density of a prey species may be economic failures because of extremely high mortality due to crabs (e.g. Mottet 1980).

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Opportunistic Cyclopoid Predation on Fish Larvae[1]

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Exceptional spring spawning of yellow perch (*Perca flavescens*) in an inland coastal lake resulted in very high larval fish densities (up to 41 900/1000 m^3) and facultative predation by adult female *Diacyclops thomasi* on newly hatched larvae (maximum predation density: 8740/1000 m^3), as evidenced by cyclopoid attachment to fish larvae in field collections. This predation suggests that larval fish mortality and possibly cyclopoid population growth are affected. Opportunistic cyclopoid predation on fish larvae may also alter zooplankton community structure by changing the feeding pattern of cyclopoids. Lack of significant predation on other species of fish larvae is probably due to temporal and spatial asynchrony of predator and prey, low probability of encounter, robust nature of certain fish species, and effective swimming and escape ability.

À la suite d'une ponte exceptionnelle de perchaudes (*Perca flavescens*) dans un lac côtier intérieur, la densité des larves de poisson a été élevée (jusqu'à 41 900/1000 m^3). La prédation facultative par des *Diacyclops thomasi* adultes sur les larves nouvellement écloses a été également élevée (densité de prédation maximale : 8740/1000 m^3), comme le démontre le degré de fixation des cyclopoïdes sur les larves dans les échantillons prélevés sur le terrain. Cette prédation laisse supposer que la mortalité des larves de poisson et peut-être aussi la croissance des populations de cyclopoïdes sont affectées. Cette prédation opportuniste des cyclopoïdes sur les larves de poisson peut également modifier la structure des communautés zooplanctoniques en modifiant les habitudes alimentaires des cyclopoïdes. S'il n'y a à peu près pas de prédation sur les larves d'autres espèces de poissons, c'est probablement à cause de l'asynchronisme temporel et spatial des prédateurs et des proies, de la faible probabilité de rencontre, de la robuste nature de certaines espèces de poissons et de l'efficacité de nage et d'évitement des proies.

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Most predaceous cyclopoids are euryphagous (Hutchinson 1967). Their prey include cyclopoids, calanoids, cladocerans, dipteran larvae, other arthropods, rotifers, oligochaetes, flatworms, and young urodeles and fishes. Recent investigations emphasize the impact of cyclopoid predation on zooplankton (Kerfoot 1977, 1978; Zaret 1980). Very little is known about the effects of cyclopoid predation on nonzooplankton.

Our initial study documented cyclopoid predation on alewife (*Alosa pseudoharengus*) and spottail shiner (*Notropis hudsonius*) larvae in southeastern Lake Michigan when cyclopoids and small fish larvae occurred together (Hartig et al. 1982). Presented here are field observations that further delineate which larval fishes are vulnerable to cyclopoid predation, the circumstances under which opportunistic cyclopoid predation on fish larvae can occur, and a discussion of potential effects of such predation that we hope will stimulate further research.

Study Area

Data presented in this paper were collected as part of an environmental surveillance effort at the J. H. Campbell Power Plant on eastern Lake Michigan (latitude 42°53′, longitude 86°29′). The plant borders a very productive inland coastal lake (Pigeon Lake) from which cooling water is drawn causing a reversal of the natural flow of Pigeon Lake into Lake Michigan, except during spring high water periods. Cooling water is discharged into Lake Michigan via a canal approximately 1 km north of the confluence of Pigeon Lake and Lake Michigan. Baseline data on the physical, chemical, and biological nature of Pigeon Lake are presented in Jude et al. (1981a).

The fish fauna of the Lake Michigan portion of our study area is diverse, with 48 species reported (Jude et al. 1980). Major species include alewife, rainbow smelt (*Osmerus mordax*), spottail shiner, trout-perch (*Percopsis omiscomaycus*), and yellow perch (*Perca flavescens*). The fish fauna of Pigeon Lake is even more diverse, with 71 species reported (Jude et al. 1981a). Of these, 49 species are considered indigenous to the Pigeon Lake–Pigeon River system, while 16 are considered as originating from Lake Michigan. Major species include alewife, spottail shiner, golden shiner (*Notemigonus crysoleucas*), yellow perch, bluntnose minnow (*Pimephales notatus*), and largemouth bass (*Micropterus salmoides*). Further information on adult, juvenile, and larval fish populations in the vicinity of the J. H. Campbell Plant is presented in Jude et al. (1980, 1981b).

Sampling Program

Fish larvae, arbitrarily defined as any fish less than 25.4 mm total length (TL), were collected using a 0.5-m-diameter, 363-μm-mesh net equipped with a calibrated flowmeter at 15 stations in Lake Michigan and 5 stations in Pigeon Lake. Duplicate hand-towed (61 m) surface samples were collected once during the day and once at night at three beach stations in Lake Michigan (once in April, May, and September and twice in June, July, and August) and two beach stations in Pigeon Lake (once in April, May, September, October, and November and twice in June, July, and August 1979). At 12 open-water stations in Lake Michigan and 3 in Pigeon Lake, 5-min horizontal fish larvae tows were performed at discrete depth strata parallel to shore on the same schedule as other tows. Samples were collected at 2-m depth intervals at stations 9 m or less, at 3-m depth intervals at stations 10–12 m, and at 4-m depth intervals at stations 15 m.

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Bottom tows were performed with a benthic fish larvae sled equipped with a flowmeter (Yocum and Tesar 1980). A single, 5-min sled tow was performed once during the day and once at night at all sampling stations in Lake Michigan. No sled tows were performed in Pigeon Lake. All samples were immediately preserved with 10% formaldehyde solution.

Fish larvae were removed from samples with the aid of a binocular microscope, identified using a preliminary draft of Auer (1982), and measured to the nearest 0.1 mm TL. Cyclopoid attachment to fish larvae was recorded as an indicator of predation. Species, developmental stage of cyclopoid, and number of cyclopoids attached were recorded. Further details on sampling methodology can be found in Jude et al. (1980).

Results

Alewife, yellow perch, spottail shiner, and rainbow smelt larvae were seasonally abundant in Lake Michigan and Pigeon Lake during 1979 (Table 1). The cyclopoids *Diacyclops thomasi* and *Acanthocyclops vernalis* were found attached to six species of fish larvae in Lake Michigan and five species in Pigeon Lake. Usually one predaceous cyclopoid was found attached to each specimen; however, there were a few specimens where two cyclopoids were attached to a larva.

Eighty-three percent of the cyclopoid predation occurred on yellow perch collected from Pigeon Lake in May. Exceptional yellow perch reproduction occurred in Pigeon Lake, as evidenced by unusually high densities (up to 41 900/1000 m^3) of yellow perch larvae in May. In addition, the catch of young-of-the-year yellow perch in 1979 was almost twice the combined 1977–78 catch (Jude et al. 1980). As a result, larvae were found at all five stations (Fig. 1 and 2), but cyclopoid predation on yellow perch larvae only occurred at four. Larvae collected from beach station S, through which Lake Michigan water passes, exhibited no predation. Highest May density of yellow perch larvae was found at 0.5 m at station X; maximum predation also occurred at this station (8700 yellow perch/1000 m^3). Higher night densities than day densities of yellow perch larvae were found at 1- to 2-m stations X, V, and S. Ninety-seven percent of all predation found in this study occurred in night collections.

All cyclopoids found preying on fish larvae were adult females. *Diacyclops thomasi* accounted for 92% of the predation. Predation in May, which accounted for 89% of the total predation, was exclusively by *D. thomasi*. Predation by *D. thomasi* also occurred in June and August, while *A. vernalis* predation occurred in June, July, and August.

Cyclopoids preyed on very small, mostly newly hatched fish larvae (Fig. 3). Yellow perch larvae preyed on by cyclopoids (mean length 6.5 mm, $N = 247$) were similar in size to those collected in the field (mean length 6.4 mm, $N = 1528$). Newly hatched yellow perch larvae range from 3 to 5 mm (Auer 1982). Mean lengths of alewife and crappie (*Pomoxis* spp.) larvae preyed on by cyclopoids were 5.1 mm ($N = 21$) and 4.5 mm ($N = 16$), respectively.

Besides alewife, spottail shiner, yellow perch, rainbow smelt, and *Pomoxis* spp. (Table 1), another 17 species and groups (485 larvae) were collected at Pigeon Lake and Lake Michigan stations. Among these, five larvae had attached copepods. In Pigeon Lake, *D. thomasi* preyed on a gizzard shad (*Dorosoma cepedianum*) and a johnny darter (*Etheostoma nigrum*) at night on 14–16 May; *A. vernalis* attacked a damaged larva at night on 16 July. In Lake Michigan, a *D. thomasi* attacked a gizzard shad larva during the day on 2 August and an *A. vernalis* was attached to a damaged larva during the night on 18 July.

Discussion

In nature, prey density is one of the primary components regulating the hunger state of a predator (Williamson 1980). High prey densities result in an increase in encounter frequency between predator and prey, but this increase in encounter frequency can only lead to increased feeding if prey are vulnerable to attack, capture, and ingestion. Both behavioral and morphological characteristics of prey are important in regulating the predator's ability to capture and ingest prey. Li and Li (1979) have demonstrated from laboratory experiments of prey selection by *A. vernalis* that body shape, carapace hardness, and mode of swimming are major influences on the selective processes. Zooplankton prey that were slow swimmers and had soft bodies and narrow body shapes were readily selected. A number of laboratory studies (reviewed in Hartig et al. 1982) document cyclopoid predation on fish larvae.

The data presented in this study show that a number of different species of fish larvae, mostly newly hatched, can be vulnerable to cyclopoid predation. We feel that these observations of predaceous cyclopoids attached to larval fishes represent natural predation (not net induced, Hartig et al. 1982) that results eventually, if not immediately, in larval fish mortality. From laboratory studies of predation by marine copepods on fish larvae, Lillelund and Lasker (1971) have noted that young pelagic fish larvae are particularly susceptible to predaceous copepods because they have an extremely thin skin and are unable to survive once the skin is punctured.

Yellow perch reproduction in Pigeon Lake was exceptional in 1979, resulting in fish larvae densities exceeding 41 000/1000 m^3 at 0.5-m station X in May. These high densities of yellow perch larvae provided *D. thomasi* a unique opportunity for facultative predation. Adult female *D. thomasi* were able to shift their feeding behavior to take advantage of newly hatched yellow perch larvae. Predation might not have occurred on larger fish larvae because increased swimming and escape ability develop as larvae grow older (Lillelund and Lasker 1971). We feel that the high incidence of *D. thomasi* predation on yellow perch larvae in Pigeon Lake (maximum predation: 8700/1000 m^3) points out a previously little-recognized source of mortality with potentially significant implications for year-class strength of yellow perch that warrants further study. From laboratory experiments and pond observations, Sukhanova (1965) determined that *A. vernalis* predation significantly affected survival

TABLE 1. Total number of various species of larval fish captured and those with attached
Lake Michigan (LM), 1979. Species of predator (D = *D. thomasi*, A = *A. vernalis*) and n
catch.

Species	Area	18–20 Apr.		14–16 May		4–5 June		20 J
		Day	Night	Day	Night	Day	Night	Day
Alewife	PL	0	0	0	0	1	0	5
	LM	0	0	0	5	11	4	39
Spottail shiner	PL	0	0	2	2	1	2	3
	LM	0	1	9	7	1	9	17
Yellow perch	PL	0	0	379 (D-2)	1149 (D-242)	4	27 (D-1)	5
	LM	0	0	97	38 (D-3)	1	1	9
Rainbow smelt	PL	0	0	8	9	1	3	2
	LM	0	0	30	30	25	33 (D-1)	115
Pomoxis spp.	PL	0	0	13 (D-1)	84 (D-15)	2	3	0
	LM	0	0	1	4	0	1	0

attached copepods at a number of pooled stations in Pigeon Lake (PL) and
s) and number of larvae attacked are given in parentheses below the total

20 June		3–4 July		16–19 July		2 Aug.		20–24 Aug.		17 Sept.	
Day	Night	Day	Night	Day	Night	Day	Night	Day	Night	Day	Night
5	26	23	15	747	695 (A-11)	106	249	300	126	1	8
39	41 (D-1)	94	130	258	36 (A-1)	524 (D-1) (A-2)	506 (D-1) (A-1)	257 (D-1)	1644 (D-1) (A-1)	108	152
3	103	0	4	8	88 (A-1)	7	457	0	13	0	6
17	41 (D-1)	6	5	91	100 (A-2)	322	408 (A-1)	1	30 (D-1)	0	0
5	11	0	0	0	0	0	1	0	0	0	0
9	24 (A-1)	10	33	2	6	1	2	2	1	0	0
2	2	0	0	0	2	0	0	0	0	0	0
115	192 (D-1)	17	15	2	2	2	1	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0

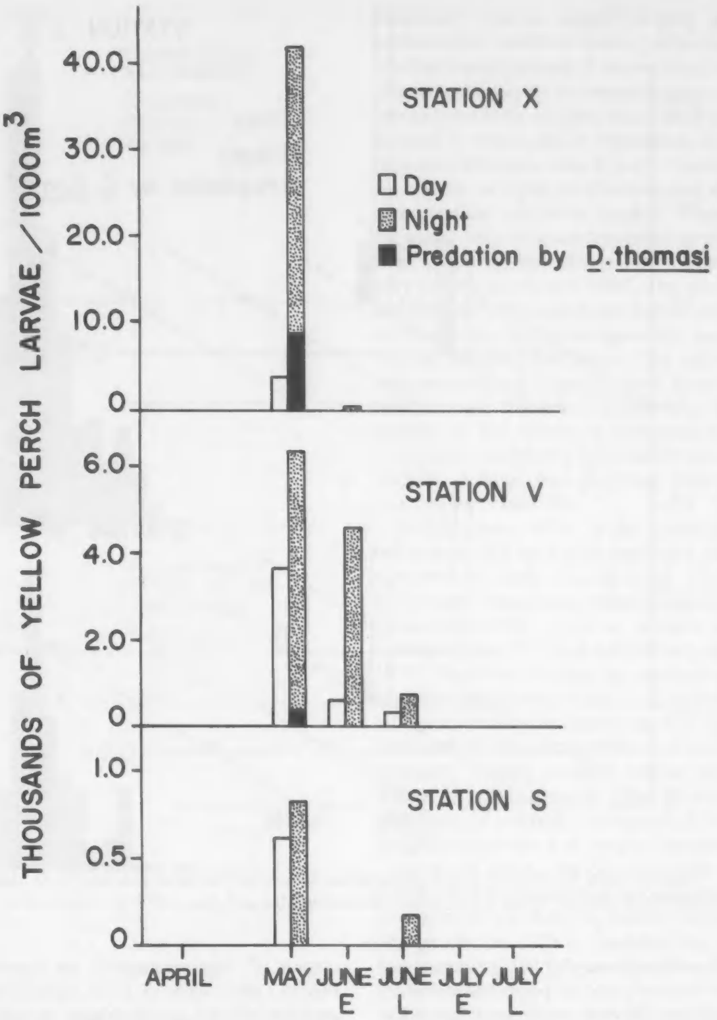

FIG. 1. Seasonal field densities of yellow perch larvae with density of those attacked by *D. thomasi* at stations X, V, and S in Pigeon Lake during May, June, and July, 1979. E = early, L = late.

rates of white carp (*Hypopthalmichthys* spp.) larvae cultured in Asia.

Based on comparable spawning times of yellow perch (Jude et al. 1981b) and identical sampling techniques and units of effort, we found much higher cyclopoid predation on yellow perch larvae in 1979 (249 incidences) than in 1978 (14 incidences, Jude et al. 1981a). This is probably due to, at least in part, less successful reproduction of yellow perch in 1978 resulting in fewer larvae in Pigeon Lake (512 yellow perch larvae were collected in May 1978, while 1518 yellow perch larvae were collected in May 1979). The reason for the difference between yellow perch larvae densities in 1978 and 1979 is not known.

In analyzing factors determining population levels and changes, Neave (1953) proposed the terms "compensatory" to describe mortality that becomes more severe as populations increase in size, "depensatory" to describe mortality that becomes more severe as populations decrease in size, and "extrapensatory" to describe mortality that is independent of size. Assuming young, pelagic fish larvae are unable to survive once their extremely thin skin is punctured, as Lillelund and Lasker (1971) have noted, then *D. thomasi* switching its feeding to abundant, newly hatched yellow perch is compensatory. Further evidence that *D. thomasi* predation on yellow perch larvae is compensatory is that the percentage of yellow perch larvae preyed upon by cyclopoids in all Pigeon Lake collections increased from 2.7 in 1978 (14 larvae preyed upon, 512 collected) to 16.4 in 1979 (249 larvae preyed upon, 1518 collected). Solomon (1949) believes that a fundamental reason for density-dependent (compensatory) predation on a given species is the limited capacity of the ecosystem with respect to that species or with respect to its enemies or both. This compensatory mortality of yellow perch in Pigeon Lake is in contrast with the depensatory mortality of yellow perch in

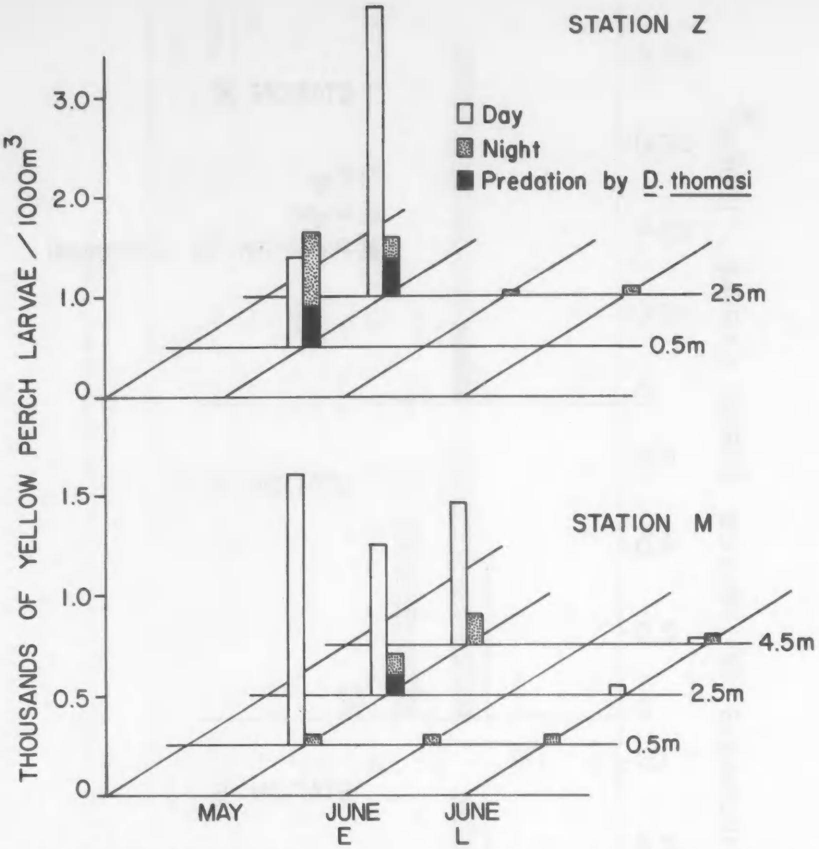

FIG. 2. Seasonal field densities of yellow perch larvae with density of those attacked by *D. thomasi* by depth stratum at stations M and Z in Pigeon Lake during May and June, 1979. E = early, L = late.

Oneida Lake, New York, where Forney's (1971) estimates of mortality based on catch in trawls and of perch consumed by walleye (*Stizostedion vitreum vitreum*) confirmed that weak year-classes generally experienced higher mortality than strong year-classes.

To a lesser extent, *D. thomasi* and *A. vernalis* also appeared to take advantage of newly hatched crappie and alewife larvae. Even though incidents of predation on these species seem low, it must be remembered that these young larvae are clearly vulnerable for at least 2 wk.

Lack of significant predation on species of fish larvae other than yellow perch, alewife, and crappie is probably due to temporal and spatial asynchrony of predator and prey, low probability of encounter (low predator and/or prey densities), robust nature of certain species such as common carp (*Cyprinus carpio*) and sculpin (*Cottus* spp.), and effective swimming and escape ability.

As noted in our previous study (Hartig et al. 1982), cyclopoid predation on fish larvae does not occur until adult cyclopoids and susceptible young fish larvae occur together. Cyclopoid predation on yellow perch and crappie larvae occurred almost exclusively in May in Pigeon Lake, while cyclopoid predation on alewife larvae occurred in July in Pigeon Lake and in June, July, and August in Lake Michigan. Yellow perch and black crappie (*P. nigromaculatus*) are known to spawn in spring in Pigeon Lake, while in 1979, alewife spawning was depressed until late July in Lake Michigan, probably the result of a summer upwelling that occurred in the nearshore area that year (Jude et al. 1980; Heufelder et al. 1982).

We found *D. thomasi* preying on newly hatched fish larvae from May to August. Predation in May occurred exclusively in Pigeon Lake due to early yellow perch spawning at water temperatures of 12–14°C (Jude et al. 1980). *Acanthocyclops vernalis* predation occurred in Lake Michigan during June, July, and August and only in July in Pigeon Lake. The delayed predation in Lake Michigan, compared with Pigeon Lake, is expected because indigenous fishes normally spawn later in Lake Michigan (Jude et al. 1980). In addition, a summer upwelling occurred in Lake Michigan in 1979 that delayed fish spawning (Heufelder et al. 1982) and possibly maturation of *A. vernalis*. This delayed spawning resulted in later cyclopoid predation on alewife larvae in eastern Lake Michigan (8 of 10 incidents of cyclopoid predation on alewife occurred in August 1979) compared with our previous study (Hartig et al. 1982), which found 99% of cyclopoid predation on alewife in July 1975–76 in southeastern Lake Michigan.

The ability of *D. thomasi* and *A. vernalis* to sense and attack moving objects, to reorient during pursuit, to repeat attacks

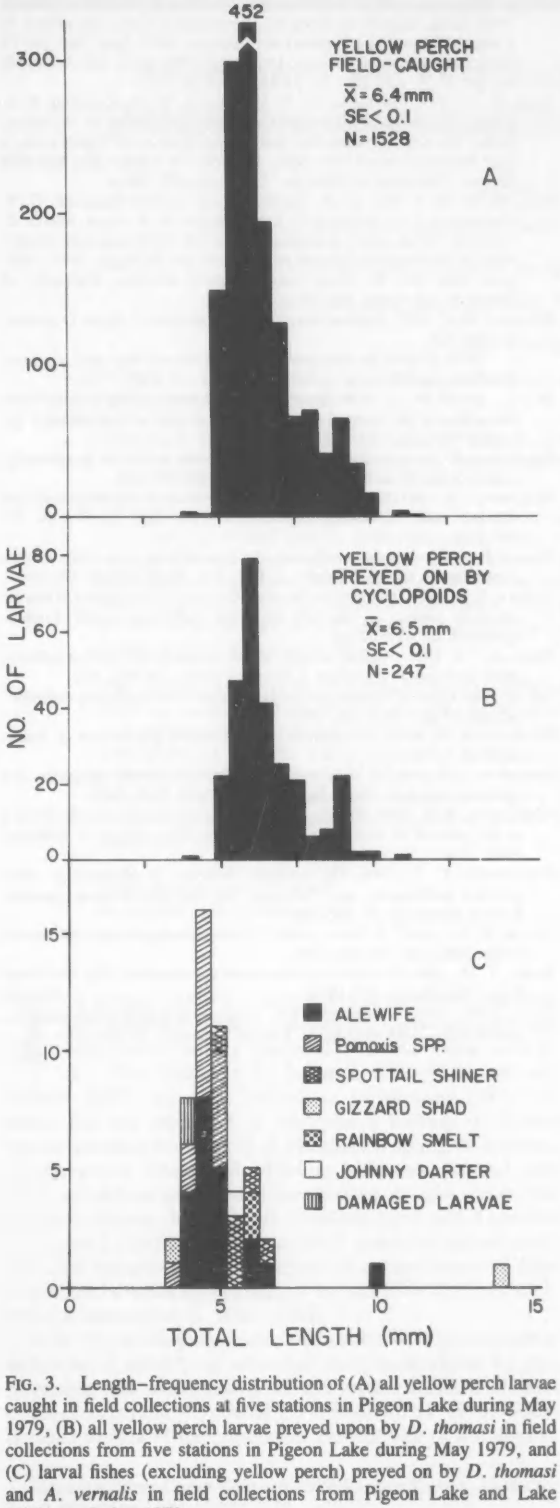

FIG. 3. Length–frequency distribution of (A) all yellow perch larvae caught in field collections at five stations in Pigeon Lake during May 1979, (B) all yellow perch larvae preyed upon by *D. thomasi* in field collections from five stations in Pigeon Lake during May 1979, and (C) larval fishes (excluding yellow perch) preyed on by *D. thomasi* and *A. vernalis* in field collections from Pigeon Lake and Lake Michigan during 1979.

tenaciously, and to reposition prey argues for a somewhat sophisticated selective feeding behavior (Kerfoot 1978). Kerfoot has found that both *D. thomasi* and *A. vernalis* can perceive objects (probably by mechanoreceptors) at a distance of at least two to three body lengths. Once they perceived their prey, the decision to attack, attack orientation, and speed of approaching prey are evaluated within 0.5–1 s. These predaceous cyclopoids are capable of rapid acceleration and sustained bursts of great speed and can maneuver quickly. Their massive first maxillae are armed with stout asymmetrical spines that can grasp prey as large as chironomids (Fryer 1957). They are known to eat their prey bite by bite (Zaret 1980). Our predation data have shown that predatory cyclopoids can handle prey as large as fish larvae and that after exceptional spawning success by fish, they may even be selecting fish larvae. This is in contrast with the belief that predatory cyclopoids avoid larger prey (Kerfoot 1977). Strickler and Twombly (1975) have speculated that capture success is not strictly a size-related phenomenon because cyclopoids use sensory information that relates to both size and velocity. A large, slow prey may present the same information as a smaller, faster one.

In this study, 97% of the predation was found in night collections. All cyclopoid predation on fish larvae reported in our previous study (Hartig et al. 1982) was found in night collections. *Diacyclops thomasi* is considered a limnetic species (Edmonson 1959), while *A. vernalis* is considered epibenthic and undergoes nocturnal vertical migration (Evans and Stewart 1977). Nocturnal feeding by predaceous cyclopoids may have adaptive significance because cyclopoids can avoid being eaten by visual-feeding fish during the day (Zaret and Suffern 1976). McLaren (1963) has hypothesized that an animal that does all necessary feeding in warm surface waters and rests in cooler waters (e.g. *A. vernalis*) gains an energy bonus. Larval fish avoidance of predatory cyclopoids during the day may also explain the relatively low levels of predation by *D. thomasi*.

In recent years, feeding has been viewed as a behavior molded by natural selection into an optimal state (Sih 1980). As food availability changes, feeding behavior of zooplankton can change (Poulet 1978). Opportunistic cyclopoid predation on fish larvae implies that cyclopoids are benefiting in some way from the predation.

Although we have no data on actual feeding rates or population dynamics, it is interesting to speculate on the adaptive significance of cyclopoid predation on fish larvae. In this study, prolific spawning of yellow perch in Pigeon Lake (Jude et al. 1980) resulted in extremely high densities of larvae, which provided *D. thomasi* a unique opportunity for predation. At these yellow perch larvae densities, predatory cyclopoids may maximize their net energy intake by eating the extremely abundant prey of high caloric value. All cyclopoids found preying on fish larvae were adult females that may have been optimizing their foraging behavior to achieve maximum net energy intake and thus gain an energy bonus for reproduction. Purcell (1981) has demonstrated a huge population growth potential of siphonophores (*Rhizophysa eysenhardti*) that feed exclusively on fish larvae of high caloric value. Possible reasons why no males preyed on fish larvae are that males are smaller than females, which may affect their ability to handle fish larvae, males may not eat as much as females because males are short-lived, and in reproduction the burden of mate detection and recognition is on males, and females are apparently sexually receptive for only a short time after reaching maturity (Gerristen 1980).

We have presented field observations of cyclopoids attached to fish larvae as evidence of opportunistic cyclopoid predation. Heavy predation by *D. thomasi* on exceptionally abundant newly hatched yellow perch larvae suggests that a zooplankter may be controlling the relative abundance of a vertebrate predator. In addition, opportunistic cyclopoid predation on fish larvae may also affect cyclopoid population growth and alter zooplankton community structure by changing the feeding pattern of cyclopoids. Further work is needed to advance our understanding of the predatory nature of cyclopoids and their impact on larval fishes as well as the plankton community.

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Abundance and Distribution of Humpback Whales (*Megaptera novaeangliae*) in West Greenland Waters

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Using photoidentification of individual humpback whales (*Megaptera novaeangliae*) and mark–recapture calculations in 1981 (mark) and 1982 (recapture), the population of this species that occurs in summer along the West Greenland coast between 63 and 70°N was estimated to be less than 200 whales. Whale distribution, however, was not random. Humpbacks were found clustered on Fyllas Banke (Godthaab District) during July and August in both years. Only a few individuals were scattered within a 240-km radius of this location. No West Greenland humpbacks have been seen in other known western North Atlantic feeding areas and vice versa.

On a estimé à moins de 200 l'effectif de la population de jubartes (*Megaptera novaeangliae*) fréquentant la côte du Groenland occidental en été, entre 63 et 70° de latitude N. Cette estimation est fondée sur l'identification par photos de jubartes individuelles et sur des calculs de marques–recaptures en 1981 (marques) et en 1982 (recaptures). La distribution de ces baleines n'est toutefois par aléatoire. On a observé des groupements de jubartes sur le Fyllas Banke (district de Godthaab) en juillet et août des deux années. Seulement quelques sujets étaient éparpillés dans un rayon de moins de 240 km de cet endroit. Aucune jubarte du Groenland occidental n'a été vue dans d'autres aires d'alimentation de l'Atlantique nord-ouest, et vice versa.

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The current size of the western North Atlantic population of humpback whales (*Megaptera novaeangliae*) has been conservatively estimated by visual census work in the West Indies to be between 2000 (Balcomb and Nichols 1982) and 4100 individuals (Whitehead 1982). Of these, 230 are estimated to comprise a feeding stock that regularly returns to the Gulf of Maine each spring (S. Katona, College of the Atlantic, Bar Harbor, ME, pers. comm.), and 1955 are estimated to comprise another distinct stock that regularly returns to feed off Newfoundland and Labrador (Whitehead 1982). In 1981, a third group of an estimated 100–200 humpbacks was documented on the banks of West Greenland between approximately 64 and 69°N (Perkins et al. 1982; Whitehead et al. 1982, 1983).

With the development and application of a photographic technique of identifying individual humpback whales by pigmentation and scar patterns on the ventral surfaces of their flukes (Perkins and Whitehead 1977; Katona et al. 1979, 1980), detailed studies of the population and ecology of this species have become possible. As of spring 1983, fluke pictures of approximately 2300 individual humpback whales have been catalogued (S. Katona, pers. comm.).

Mark–recapture population estimates, based on fluke photographs of individuals, have been used to assess the size of the summer feeding stocks and the total Northwest Atlantic population of humpback whales by Perkins and Whitehead (1977), Whitehead (1982), and Whitehead et al. (1982, 1983). These techniques are particularly useful for estimating the abundance of humpback whales because their natural "tags" (fluke patterns) are unique to each individual and appear to be stable over time (at least 14 yr). The precision of different statistical methodologies related to the use of mark–recapture estimators for these populations has been reviewed by Whitehead (1982).

Perkins et al. (1982) sighted 25 humpbacks along the West Greenland coast in the summer of 1981, 13 of which were photoidentified. Later that summer, Whitehead et al. (1982) obtained photographs of an additional 60 humpbacks off the west coast of Greenland. We considered 9 photographs from this total of 73 (13 + 60) to be inadequate in quality to use for reliable reidentification, and therefore the usable total is 64 photoidentified humpback whales for 1981. None of these 64 individuals had been previously or subsequently photographed on any other feeding grounds, but three of them had been photoidentified in southerly breeding areas: two on Silver Bank north of the Dominican Republic (one in February 1980 and the other in February 1981) and the third off Puerto Rico in 1981 (Katona et al. 1982). These findings demonstrated the use of West Indian breeding sites by at least some members of the West Greenland aggregation.

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To establish whether humpback whales identified from West Greenland return there regularly, as suggested by the return of a "Discovery" tag reported by Mitchell (1974), and to determine the summer range and population status of this aggregation, a second cruise to those waters was undertaken aboard the 44-m barkentine R/V *Regina Maris* during July and August 1982. This paper reports the findings of that 1982 expedition and mark–recapture estimates of the humpback whale population that occurs along the west coast of Greenland.

Methods

We designed our survey so that the ship's track would cover the maximum possible area on the West Greenland fishing banks during the brief summer season available. Based on sighting data (including our own in 1981), catch statistics, local sources, and ice conditions, we chose to survey the continental shelf between 63 and 70°N (Fig. 1). Our search effort (number of days and hours of observation per day) was divided fairly equally among the offshore banks between those latitudes. Storis or "east ice," which sweeps around Cap Farvel and drifts northward along the West Greenland shore, prevented a survey of coastal waters south of Frederikshaab (62°N), where some humpback whales occur and are hunted (Grønlands Fangstlister 1974; Kapel 1979).

Whenever humpback whales were encountered, they were approached and followed for a sufficient length of time to obtain fluke photographs and record their group associations and behavior. The photographs were compared with those taken in 1981 to ascertain whether any individuals had been previously photoidentified in West Greenland waters. They were also compared with other catalogued photographs of Northwest Atlantic humpback whales.

The photographs of humpback whale flukes were evaluated on a quality gradient from good (1) to poor (4), determined by image sharpness, contrast, resolution, and size of the tail in relation to the frame area of the picture. All photographs of grade 4 were deleted from the collection to minimize error and avoid duplication.

Using mark–recapture data for whales photographed in both 1981 and 1982, we employed Chapman's modification of the Petersen method (Seber 1973) to estimate the population size ($\hat{N}$) of humpback whales off West Greenland between 63 and 70°N, with 95% confidence intervals (Seber 1973, p. 60):

$$\hat{N} = \frac{(n_1 + 1)(n_2 + 1)}{(m_2 + 1)} - 1$$

where n_1 is the number of photoidentified whales in the first sample (1981), n_2 is the number of whales photoidentified in the second sample (1982), and m_2 is the number of whales photoidentified in both samples. The assumptions underlying Petersen's method (see Seber 1973) are as follows: (1) the population is closed: no whales leave or enter the population before the second sample, (2) during a sampling period, all whales have the same probability of being sighted, photographed, and identified, and (3) fluke patterns do not change between sample periods and each pattern can be identified.

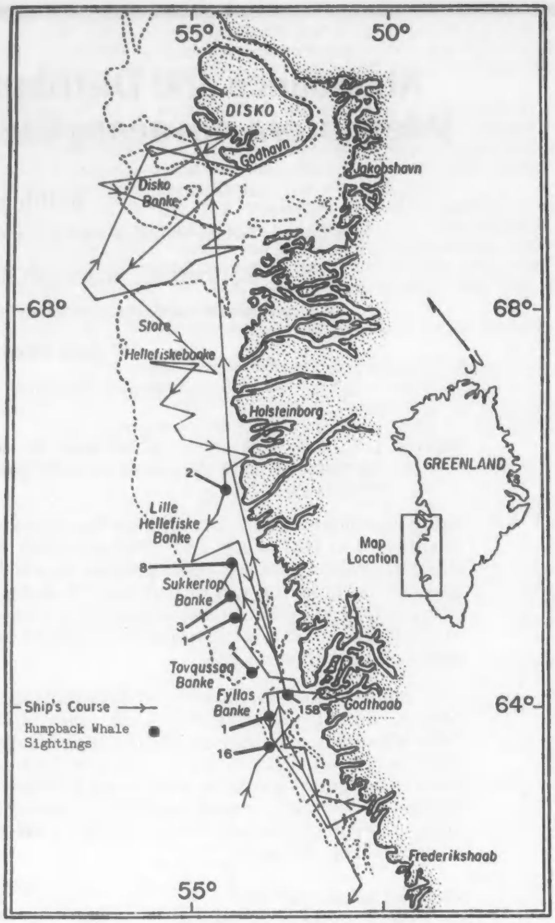

FIG. 1. Humpback whale sightings and track of R/V *Regina Maris* along the West Greenland coast in July and August 1982.

Results

Observations — Between 20 July and 28 August 1982, 193 humpback whale sightings were recorded on or near Fyllas, Tovqussaq, Sukkertop, and Lille Hellefiske Bankes between 63°26′N and 68°03′N (Fig. 1). Total "sightings" exaggerate the actual number of individual humpback whales seen because some whales were resighted on different days.

As in 1981, we found that humpback whales were not evenly distributed throughout the survey area but were concentrated off the northeast edge of Fyllas Banke. There, during 35 survey days in 1982, the majority of sightings were made between 13 and 24 August. The principal aggregation of whales seen during this 12-d period remained within a 25-km^2 area along the northeast side of Fyllas Banke. Nine calves, all in the company of adults (presumably mothers), were seen in this area. Only 18 humpback whales were found elsewhere along the West Greenland coast, principally on Lille Hellefiske, Sukkertop, and Tovqussaq Bankes, and none were seen during transects on Store Hellefiske Banke to the north or on Fiskenaes or Danas Bankes to the south of Fyllas Banke. A solo subadult whale, observed on Sukkertop Banke on 21 August (65°05′N, 53°54′W), was later observed on Fyllas Banke on 24 August (64°08′N, 52°39′W), thus had traveled a minimum of 115 km in 3 d.

We obtained useful identification photographs of 63 individual humpback whales, 56 of which were from Fyllas Banke (13–24 August). Matching fluke pictures revealed that 22 of these whales (34%) had been previously photographed there during the summer of 1981, thereby bringing the total number of photoidentified humpbacks from West Greenland to 105 (64 + 63 − 22 matches).

One humpback whale, photographed on 21 August 1982 on the southern part of Lille Hellefiske Banke, had been previously photographed 14 yr earlier in the spring of 1968 off Bermuda, resulting in the longest interval between resighting of any Atlantic humpback whale (Katona et al. 1982). With that, the total number of humpback whales known to migrate between the western Atlantic areas and West Greenland is four: two to Silver Bank (1980, 1981), one to Puerto Rico (1981), and one to Bermuda (1968). None of the photodocumented humpbacks from the west coast of Greenland match those from any of the other feeding stocks in the Northwest Atlantic (S. Katona, pers. comm.), which strongly suggests that the West Greenland aggregation is a discreet feeding stock.

Population estimate — The population estimate ($\hat{N}$) for West Greenland humpback whales between 63 and 70°N, derived from Chapman's modification of Petersen's estimator (Seber 1973), is 180, with 95% confidence limits of 137–223, assuming normality. The validity of the assumptions underlying Petersen's model is discussed below.

Discussion

Summer range — Available evidence from sighting data, catch statistics, and local sources indicates that Fyllas Banke is the principal habitat for humpback whales in West Greenland, both in terms of abundance and residence time (Kapel 1979; Perkins et al. 1982; Whitehead et al. 1982, 1983). During our 1981 and 1982 surveys, only relatively small numbers of humpback whales were seen to the north (12 in 1981 and 18 in 1982), and in both years, 2 of these animals were also photographed on Fyllas Banke. These findings suggest that large foraging movements occur, but that Fyllas Banke functions as a center of distribution for this species off West Greenland. Our data suggest that the major concentration of humpbacks moves as a semicohesive group around the edges of Fyllas Banke but resides along the northeast corner of the bank in August. Although more observations are required to support this conjecture, these herdlike movements are similar to those of humpback whales that summer on Stellwagen Bank, Gulf of Maine, where they shift to different sections of the bank in a fairly regular sequence through time (Mayo 1980).

Population estimate — Our population estimate of 180 ± 43 humpback whales in West Greenland waters between 63 and 70°N is reasonable, considering the number of whales seen and the area covered, but it is preliminary. There remains a need to quantify the biases affecting the validity of the underlying assumptions for our calculations. In both 1981 and 1982, we were unable to survey waters south of 62°N because of heavy pack ice, and therefore, we may have missed photographing some whales in both years. However, existing information on catch statistics and sightings, as summarized by Kapel (1979), suggests that relatively few humpback whales have been taken (7 of 182 between 1923 and 1976) or seen south of the Godthaab and Frederikshaab Districts. Nevertheless, more data are required to indicate how many humpback whales migrate through or remain in south Greenland waters.

Assumption "1" of Petersen's estimator (no whales leave or enter the population) was almost certainly violated, as one or more whales are likely to have died from natural causes or hunting between the sample periods. The bias introduced is proportional to the magnitude of these deaths, which in the case of natural causes is approximately 0.05–0.09/yr (Chittleborough 1965). Hunting mortality (10+ whales/yr) may further bias the estimate. In both cases, the bias would cause the population to be overestimated, and their combined effect could cause an overestimate of the population by as much as 15%. The birth of offspring, also contained in assumption 1, creates a bias in the population estimate. A precise estimate of natality is not possible, given the need to make many assumptions about the life histories of these whales (e.g. weaning age, timing of migration of mother–calf pairs relative to the rest of the population, and differences in birth rates between declining and increasing populations), all of which are not well known. The net effect of natality, however, is to dilute the marked population in the 1982 sample, thereby causing an overestimate of the total population.

Concerning assumption 2 of Petersen's estimator (all whales have the same probability of being sighted, photographed, and identified), the survey area was covered fairly evenly. Although line transect data were not collected, we consider it valid to assume that there was an equal probability of sighting each humpback if it was within 2 km of the vessel's path. Sighting proficiency, however, was affected by different weather conditions (sea state, wind velocity, and visibility). Since not all humpback whales are equally likely to fluke-up upon diving, this bias will also affect assumption 2. Mothers with calves (and the calves themselves) and solo juveniles fluke-up less regularly than other humpbacks. We are not able to precisely quantify this bias caused by individual variation in fluking behavior, but our subjective impression suggests that it will lead to an underestimate of the population by approximately 10–20%. Assumption 2 also requires that the probability of a humpback whale being photographed in the second sample (recapture) be independent of whether it was photographed in the first sample (mark). In our experience, photoidentifying an individual once does not change the lilelihood of photographing it on some subsequent occasion.

Regarding assumption 3 of Petersen's estimator (fluke patterns do not change between sample periods and each pattern can be identified), the 14-yr interval (1968–82) between resightings of one humpback whale is a prime example of the retention of fluke patterns for at least that length of time. Although we have been able to routinely reidentify individuals from fluke pictures, we do not know if natural markings change so dramatically as to prevent later reidentification. Difficulties associated with finding matching fluke photographs are primarily related to picture quality, differing angles of fluke entry into the water, and orientation of the flukes relative to the axis of the camera lens. As previously stated, poor-quality photographs were deleted to minimize errors in the photoidentification process. We cannot directly evaluate the extent or direction of any bias that may result from violation of assumption 3, but we consider it to be minimal.

Feeding stock discreetness — The absence of matching fluke photographs between Greenland and other known Northwest Atlantic feeding areas supports the hypothesis that West Greenland humpback whales constitute a discreet feeding stock. A further measure of the distinctness of these feeding stocks derives from the increasing number of photoidentified humpback whales known to return to the same feeding areas in different years. Although the proportion of identified individuals varies with relative stock size, search effort, and feeding area size, the current knowledge of the separate feeding stocks is as follows: in the Gulf of Maine, 108 of a total estimated stock of 230 whales have been photographed there in more than 1 yr (Katona et al. 1982); off Newfoundland/Labrador, 123 of an estimated stock size of 1955 humpback whales have been photographed in more than 1 yr (Whitehead 1982; Katona et al. 1982); and with our current results off West Greenland, 22 of the estimated 180 humpback whales have been photographed there in 1981 and 1982. No humpback whale photographed in any of these feeding areas has yet been photographed in any other feeding area.

Because individual whales from these different feeding stocks have been photographed off the West Indies and Bermuda during the winter breeding season, a potential gene exchange within the entire Northwest Atlantic population is possible. If this is the case, it follows that the Greenlandic subsistence harvest of 10 humpbacks/yr can be safely sustained by the breeding population as a whole. However, the question of greatest significance to Greenlanders and to the International Whaling Commission is whether the annual catch of 10 whales can be sustained by a discreet West Greenland feeding stock, numbering approximately 180 whales. The affinity of humpbacks for aggregating at traditional sites makes them particularly vulnerable to exploitation. It has been shown by Beddington and May (1982) that exploited whale populations in a state of decline do not necessarily recover when given respite from hunting pressure. This situation is partly attributed to ecological replacement by sympatric species possessing similar food preferences. To what extent these processes are applicable to West Greenland waters is unknown.

Addendum

Since the acceptance of this manuscript for publication, we have conducted a third research expedition to West Greenland in the summer of 1983. Our survey in July and August 1983 was more extensive than previous surveys, covering coastal waters from 62 to 69°N. An aggregation of about 20 humpback whales was found off Frederikshaab (62°N). Two of these whales had been photographed in previous years on Fyllas Banke, indicating that they do not represent a separate stock. The 1983 mark–recapture estimate for the entire West Greenland feeding stock of humpback whales was 282, with 95% confidence limits of 206–358.

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Accumulation and Metabolism of Tri-*n*-butyltin Cation by a Green Alga, *Ankistrodesmus falcatus*

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Tributyltin pesticides are increasingly widespread, but their environmental persistence is poorly known. The accumulation and metabolism of tri-*n*-butyltin cation (Bu_3Sn^+) by a green alga, *Ankistrodesmus falcatus*, was determined at 20°C over a 4-wk period; about 50% of the original Bu_3Sn^+ was converted to di-*n*-butyltin (Bu_2Sn^{2+}) and small quantities of butyltin ($BuSn^{3+}$) and inorganic tin. An apparent algal bioconcentration factor of 3×10^4 was estimated for Bu_3Sn^+.

L'utilisation de pesticides à base de tributylétain est de plus en plus répandue, sans qu'on connaisse trop leur persistance dans l'environnement. L'accumulation et le métabolisme du cation tri-*n*-butylétain (Bu_3Sn^+) dans une algue verte, *Ankistrodesmus falcatus*, ont été déterminés à 20°C pendant une période de 4 sem. Environ 50 % du Bu_3Sn^+ ont été convertis en di-*n*-butylétain (Bu_2Sn^{3+}), et en petites quantités de butylétain ($BuSn^{3+}$) et d'étain inorganique.

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Organotin compounds are used in three main ways: as thermal stabilizers for polyvinyl chloride, as catalysts in the production of polyurethane foams, and as biocides (Zuckerman et al. 1978). The increasing annual usage of organotin compounds raises the possibility of environmental pollution. Organotin compounds are a chemical class about which more information is sought under Canada's Environmental Contaminants Act (Canada Department of Environment and Department of National Health and Welfare 1979) regarding toxicology and environmental fate. We have recently reported the occurrence of butyltin and methyltin species in Ontario waters (Maguire et al. 1982) and sediments (Maguire 1984). We have also examined the toxicity of a number of inorganic and organic tin compounds to pure algal cultures as well as natural phytoplankton in lake water (Wong et al. 1982). Organic tin compounds were generally more toxic than inorganic tin compounds. The toxicity of organic tin compounds varied considerably with the number and nature of the organic groups attached to tin, with trialkyltin compounds being the most toxic forms.

Concurrent with these studies is an attempt to estimate the aquatic persistence of a representative organotin biocide, bis(tri-*n*-butyltin) oxide (TBTO), by determining the relative importance of a variety of routes of degradation. TBTO was chosen because it is used in some antifouling boat paints (Zuckerman et al. 1978) and is a likely source of the Bu_3Sn^+ observed in Ontario waters. We have shown (Maguire et al. 1983) that Bu_3Sn^+ dissolved in natural water undergoes slow ($t_{1/2}$ 89 d) sunlight photolytic degradation, at least partially by stepwise debutylation to inorganic tin, and that over a period of at least 2 mo it did not volatilize, nor did it undergo reaction in the dark.

The cation Bu_3Sn^+ is fairly toxic to aquatic organisms, with LC_{50}^{24h} values of 28 $\mu g \cdot L^{-1}$ for rainbow trout (*Salmo gairdneri*) (Chliamovitch and Kuhn 1977) and 3 $\mu g \cdot L^{-1}$ for *Daphnia magna* (Polster and Halacka 1972). At sublethal concentrations, Bu_3Sn^+ could be degraded by microorganisms in soil, as well as by pure cultures of certain bacteria and fungi, to di- and mono-butyltin species (Barug and Vonk 1980; Barug 1981). Blair et al. (1982), however, noted that tin-resistant estuarine bacteria isolated from sediments of Chesapeake Bay could accumulate, but not metabolize, Bu_3Sn^+. To our knowledge, no information is available on the accumulation of Bu_3Sn^+ by algae; it was therefore of interest to determine if algae could accumulate and metabolize Bu_3Sn^+.

As discussed elsewhere (Maguire et al. 1983), the dissolution of Bu_3SnX compounds (where X =F, Cl, Br, OAc, and $OSnBu_3$, i.e. TBTO) in water probably produces the hydrated Bu_3Sn^+ ion. For brevity, the tri-*n*-butyltin, di-*n*-butyltin, and *n*-butyltin species are referred to in this article as though they existed only in cationic form (e.g. Bu_3Sn^+); we were more interested in debutylation reactions than in cation hydrolysis because the toxicity of butyltin compounds decreases with decreasing number of butyl groups (Davies and Smith 1980).

Materials and Methods

TBTO (97%), tri-*n*-butyltin chloride (97%), di-*n*-butyltin dichloride (96.5%), *n*-butyltin trichloride (95%), metallic tin (99.99%), 48% HBr, and *n*-pentylmagnesium bromide in diethyl ether were obtained from Ventron (Danvers, MA). (Three different lots of *n*-pentylmagnesium bromide purchased after completion of this work were found to contain unacceptably high concentrations of four butylpentyltin compounds, $Bu_nPe_{4-n}Sn$, and thus in our current work the Grignard reagent is synthesized from commonly available chemicals.) All butyltin compounds were purified by passage in hexane through a 60 × 1.5 cm (inside diameter) column of activated Florisil® (Fisher Scientific Co., Toronto, Ont.) and were pure, as judged by gas chromatography of their *n*-pentyl derivatives (see below) with flame photometric, flame ionization, and electron capture detectors. Tropolone (2-hydroxy-2,4,6-cycloheptatrien-1-one) was from Aldrich (Milwaukee, WI) and was recrystallized (mp 52°C, uncorr.) from diethyl ether before use. All organic solvents were pesticide grade from Caledon Laboratories (Georgetown, Ont.), and all mineral acids were Aristar grade from BDH Chemicals (Toronto, Ont.).

Algal culture — The axenic culture of *Ankistrodesmus falcatus* var. *acicularis* (Ontario Ministry of the Environment, Rexdale, Ont.) was grown in 500 mL of CHU-10 medium (Chu 1942) at 20°C on a rotary shaker (100 rpm) under conditions of 18 h of light (5000 lx) and 6 h of darkness. When the culture reached the logarithmic phase of growth (about 1 wk), it was used as inoculum.

Accumulation and degradation of Bu_3Sn^+ by algae — Five 7-L glass bottles, each containing 4 L of CHU-10 medium, were sterilized by autoclaving. Upon cooling, one bottle was labeled as the "control" for determining possible chemical degradation or photodegradation. The other four bottles were each inoculated aseptically with 100 mL of the algal culture. The culture and the medium were mixed continuously with a magnetic stirrer under a constant light intensity of 5000 lx. When the culture reached the logarithmic phase of growth (as determined spectrophotometrically), a concentrated stock solution of TBTO in methanol was added to each of the five bottles to give an initial concentration of $20\ \mu g\ Bu_3Sn^+ \cdot L^{-1}$ in the medium. After 1 wk of incubation at 20°C, 1 L of the culture was removed aseptically from each of the four test bottles. These were pooled to give a 4-L sample. One of the four experimental bottles was redistributed into the other three bottles so that the final volume of each of the three bottles was again 4 L. This procedure minimizes variation among cultures. In the 4-L sample the algae were separated from the medium by centrifugation at 16 000 × *g* for 20 min. One 500-mL sample from the control bottle was also withdrawn for analysis.

After 2 wk of incubation, approximately 1.3 L was removed from each of the three test bottles and combined. One of the three bottles was then divided evenly between the other two bottles, resulting in two bottles containing 4 L each. The algae were separated from the medium as described above, and a control sample was also withdrawn for analysis.

After 3 wk of incubation, 2-L samples were taken from each of the two bottles and the remaining samples were combined into one bottle. The algae were separated and the control medium was sampled as described above.

Finally, after 4 wk of incubation the last samples were taken as indicated above and the experiment was ended.

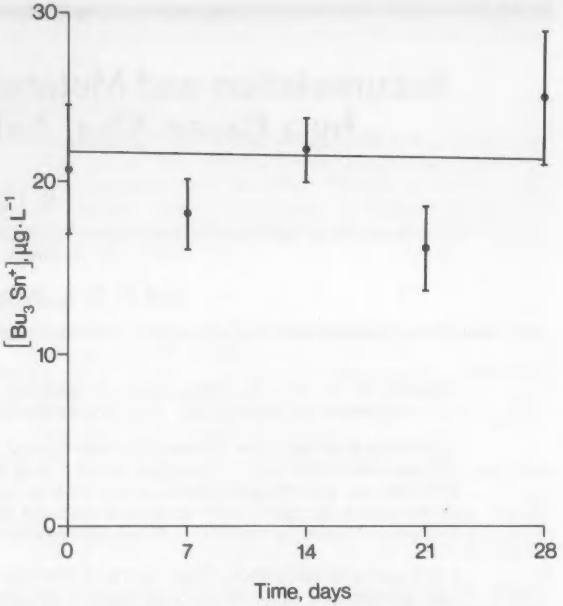

FIG. 1. Stability of Bu_3Sn^+ control in nutrient medium with time. Error bars represent standard deviations of triplicate gas chromatographic injections. Slope of the line, calculated by least-squares analysis, is $-(1.77 \pm 2.09) \times 10^{-1}\ \mu g \cdot L^{-1} \cdot d^{-1}$, which is zero within experimental error.

Measurement of algal biomass — Concurrent with the accumulation–degradation study, samples of algal suspensions were withdrawn for determination of biomass. Cells were enumerated under a microscope with a Petroff–Hausser counting chamber (Frobisher 1968) and were then related to a previously constructed dry weight curve. All cell count data were based on three separate counts of each sample. The initial cell counts of *A. falcatus* were $5 \times 10^4\ cells \cdot mL^{-1}$ (17 mg dry wt/4 L). The cell numbers increased to $2.8 \times 10^5\ cells \cdot mL^{-1}$ ($100\ mg \cdot 4\ L^{-1}$) and $4.5 \times 10^5\ cells \cdot mL^{-1}$ ($161\ mg \cdot 4\ L^{-1}$) after 1 and 2 wk of incubation, respectively. Thereafter, there was no further increase in the cell numbers with time; apparently a steady state of growth was reached. A similar observation was made for *A. falcatus* exposed to a mixture of metals (Wong et al. 1983).

Analysis of butyltin species and inorganic tin in medium and algae — Bu_3Sn^+, Bu_2Sn^{2+}, $BuSn^{3+}$, and inorganic tin were analyzed as *n*-pentyl derivatives, $Bu_nPe_{4-n}Sn$, by gas chromatography with a modified flame photometric detector (Maguire and Huneault 1981). Typically, to 25–50 mL of an aqueous solution of, or an algal suspension containing, butyltin species and inorganic tin, 3 mL of concentrated HCl and 3 mL of 48% HBr were added, and the aqueous phase was extracted with two 50-mL aliquots of 0.2% (w/v) tropolone in benzene; the benzene extract was then derivatized and analyzed as described previously (Maguire and Huneault 1981). This procedure yields quantitative recoveries for Bu_3Sn^+ (from either TBTO or Bu_3SnCl), Bu_2Sn^{2+}, and $BuSn^{3+}$ from algae, nutrient medium, or pure water at pH 1–7; although Sn^{4+} at pH 1 can be recovered quantitatively by this method, recoveries are low and variable in experiments conducted at pH 5–8, probably because unextractable SnO_2 is formed.

Although Sn^{2+} was the only inorganic tin species for which recoveries were determined, the inorganic tin present in the

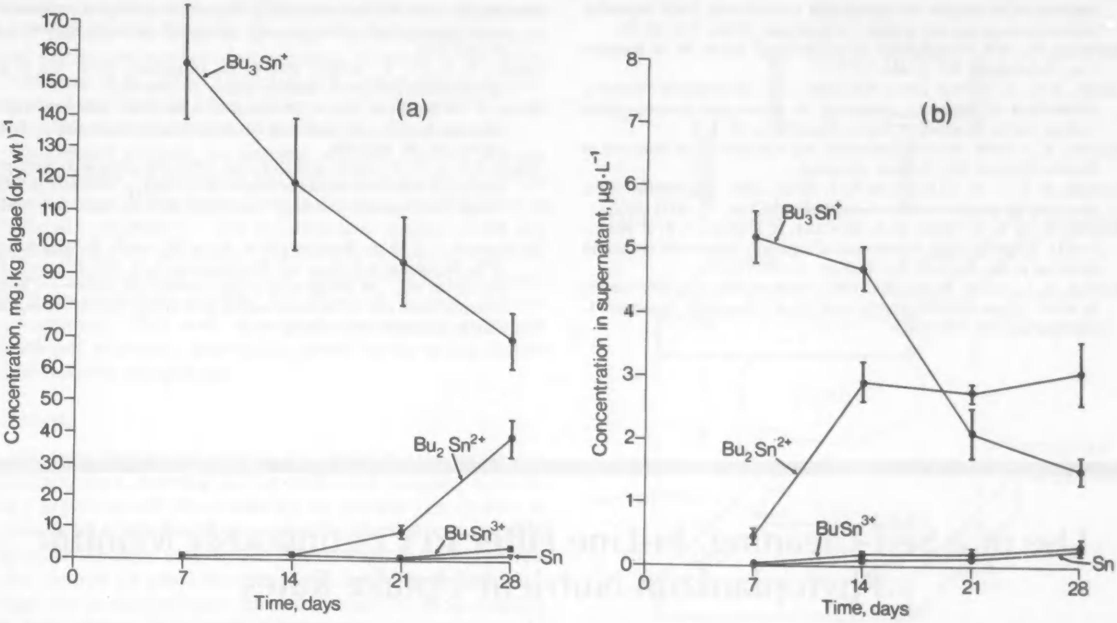

FIG. 2. Concentrations of butyltin species and inorganic tin in (a) *A. falcatus* and (b) supernatant medium from the same experiments.

samples is reported as total recoverable inorganic tin, Sn, since it has been shown that hydride derivatization of either Sn^{4+} or Sn^{2+} yields SnH_4 (Brinckman et al. 1983), and thus any Sn^{2+} that may be present in water or algae may similarly be pentylated to Pe_4Sn.

Results and Discussion

Bu_3Sn^+ in nutrient medium alone is stable under the laboratory lights over 4 wk (Fig. 1); only traces of Bu_2Sn^{2+}, $BuSn^{3+}$, and Sn were detected. On the other hand, *A. falcatus* is able to degrade Bu_3Sn^+, with the first and major product being Bu_2Sn^{2+} (Fig. 2a). In addition, small quantities of $BuSn^{3+}$ and Sn were observed. A half-life of about 25 d can be estimated from Fig. 2a for the algal degradation of Bu_3Sn^+, but this estimate should be viewed with caution, since the reaction was not followed to completion. A mass balance shows that at day 28, 83% of the Bu_3Sn^+ (calculated as milligrams of tin) present at day 7 could be accounted for either as starting material or products. The concentrations of the butyltin species and inorganic tin in the supernatant medium after centrifugation (Fig. 2b) mirror the trends in the algae. These results are in agreement with a scheme of sequential debutylation, hence detoxification, of Bu_3SnX compounds proposed for metabolism in vitro with rat liver microsomal enzymes (Fish et al. 1976) and in vivo by mice (Kimmel et al. 1977). The possibility of Bu_3Sn^+ metabolism by bacterial contamination of our algal suspensions is ruled out, since the algal culture was free of bacteria and the experiment was carried out under aseptic conditions.

A maximum apparent algal bioconcentration factor of 3×10^4 is estimated for Bu_3Sn^+ at day 7, since there appeared to be little degradation of Bu_3Sn^+ (cf. Fig. 2a and 2b) at that time. In another experiment in which the algae were exposed to Bu_3Sn^+ at a higher concentration ($40\,\mu g \cdot L^{-1}$), the apparent algal bioconcentration factor was 8.6×10^3. Seidel et al. (1980), in an extensive survey of tin pollution in the marine environment, found that marine algae were able to accumulate Sn^{4+} by a factor of 1.9×10^3 over water. Several tin-resistant estuarine bacteria were reported to accumulate Bu_3Sn^+ to 3.7–7.7 mg Sn·g cells (dry wt)$^{-1}$ (Blair et al. 1982).

The persistence of Bu_3Sn^+ in natural waters vis-à-vis algal degradation will be a function of temperature and the kinds and concentrations of Bu_3Sn^+-degrading algae, and their nutritional state, so it is difficult to assess whether algal degradation of Bu_3Sn^+ would be faster than photolysis in situ (see above). Further work is in progress on Bu_3Sn^+ adsorption to sediments, bacterial degradation, and uptake and metabolism by trout.

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Use of a Self-Cleaning, In-Line Filter to Continuously Monitor Phytoplankton Nutrient Uptake Rates

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Our new technique allows the decline in ambient ammonium concentration to be followed continuously during phytoplankton uptake experiments. A self-cleaning, in-line filter permits the continuous separation of cells from the medium for nutrient determination by an autoanalyzer. The technique works well with laboratory cultures of the marine diatoms *Thalassiosira pseudonana* and *Skeletonema costatum*. Changes in uptake rate on time scales of 1 min or longer can be resolved, the resolution being limited by the response characteristics of the autoanalyzer.

La nouvelle technique décrite dans le présent article permet de suivre de manière continue la diminution de la concentration d'ammoniac ambiant au cours d'expériences sur la captation par le phytoplancton. Grâce à un filtre autonettoyeur inséré sur la conduite, il y a séparation continue des cellules et du milieu de culture, permettant à une auto-analyseur de doser les substances nutritives. Cette méthode donne de bons résultats avec des cultures en laboratoire des diatomées marines *Thalassiosira pseudonana* et *Skeletonema costatum*. Des changements de taux de captation peuvent être détectés à échelles temporelles de 1 min ou plus, les caractéristiques de réaction de l'auto-analyseur limitant le pouvoir de résolution.

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The uptake of a nutrient by phytoplankton can be detected either as the disappearance of the nutrient from the medium or as the incorporation of a labeled form of the nutrient (e.g. ^{15}N, ^{32}P). Early studies measuring disappearance from the medium calculated an average uptake rate between nutrient determinations at the beginning and end of a single incubation, usually several hours in duration (e.g. Eppley et al. 1969). Such long incubations seemed reasonable because it was generally accepted that each phytoplankton species or clone had an uptake rate that depended only on the ambient concentration of the nutrient in question; rapid changes in uptake rate were not suspected.

It is now known that nutrient uptake rates are closely coupled to a culture's physiological status. In particular, when a nutrient-limited or starved population is suddenly exposed to high concentrations of the limiting nutrient, the cells' internal state and the uptake rate can change rapidly, on time scales of minutes or even seconds (Conway et al. 1976; McCarthy and Goldman 1979; Goldman and Glibert 1982). Therefore, a population's uptake kinetics cannot be characterized by measurements over a single incubation interval because the average uptake rate will change with the interval chosen. To follow rapid changes in uptake rates, a large number of discrete subsamples must be taken at frequent intervals for immediate filtration and subsequent nutrient determination (Conway et al. 1976; DeManche et al. 1979; Goldman and Glibert 1982). Experiments of this kind are labor intensive and require care to avoid contamination in the handling and filtration of subsamples.

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Automated systems for nutrient analysis monitor nutrient concentrations continuously and should in principle allow the disappearance of external nutrient in an uptake experiment to be followed continuously. The instantaneous uptake rate at any time can then be calculated as the negative slope of a plot of nutrient concentration versus time. This approach offers superior time resolution, without the effort of frequent discrete subsampling. This note describes a continuous technique developed to monitor ammonium uptake by the marine diatom *Thalassiosira pseudonana*.

Methods

Whereas discrete subsamples are filtered to stop uptake at a particular time, filtration during continuous sampling is necessary to prevent cells from entering the autoanalyzer system. In preliminary experiments, we established that cells encountering the harsh chemical and physical environment of the indophenol blue method for ammonium determination (Slawyk and MacIsaac 1972) released their internal ammonium pools, causing an overestimate of the ambient ammonium concentration. An obvious solution was to insert an in-line filter between sample and autoanalyzer to remove cells. However, cells accumulate on an in-line filter and continue to take up ammonium from the medium passing across the filter, again distorting the uptake estimates.

In-Line Filter

The key to this implementation of the continuous approach has been the development of an in-line filter system that will not collect cells (Fig. 1 and 2). The filter is clamped between two Plexiglas squares, 7 cm × 7 cm × 9 mm deep (Fig. 1). Matching channels 2 mm wide, 1.5 mm deep, and 3 cm long are cut in the surfaces in contact with the filter. The culture enters the upper channel through the inlet hole A. Part of the inflow (approximately $1.6\,\mathrm{mL \cdot min^{-1}}$) is drawn through the filter, along the lower channel as cell-free filtrate, and through outlet C by the autoanalyzer pump. The remainder of the inflow (approximately $0.8\,\mathrm{mL \cdot min^{-1}}$) is drawn along the upper surface of the filter and through outlet B by the pump, to be eventually discarded as waste. A flow along the top of the filter will not by itself dislodge cells held against the filter by the pressure drop across it. These cells can only be dislodged if the pressure drop is removed or reversed. A pressure reversal is supplied by squeezing a flexible, 3-mm (i.d.) silicon section of the filtrate outflow tubing (Fig. 2). Since additional fluid cannot be forced past the peristaltic pump, the filtrate is forced back through the filter, flushing cells off it in the process. The excess, together with the cells flushed off the filter, is blocked by the one-way valve V_A and forced through the overflow valve V_B. Again, this excess cannot be forced past the pump, so a sealed, air-filled tube D is used to provide a temporary reservoir between the value V_B and the pump. As the silicon tube is released, additional medium is drawn through the intake and across the filter, while the one-way value V_B prevents waste in D from being pulled back onto the filter.

A solenoid and timer are used to provide pressure reversals at 10-s intervals, limiting cell accumulation to 10-s periods where its effect on nutrient uptake is negligible. The effectiveness of the self-cleaning procedure can be checked visually, since accumulating cells form a green film on the filter. It can also be checked by running a known ammonium standard with the filter in line, since accumulated cells take up ammonium and lower the standard peak height. The flushing technique works efficiently as long as the porosity of the filter remains high. Using unialgal laboratory cultures of *T. pseudonana*, a 1-μm pore size Nucleopore® polycarbonate filter will remain clean for several hours. The filter can be replaced between uptake experiments in a few minutes.

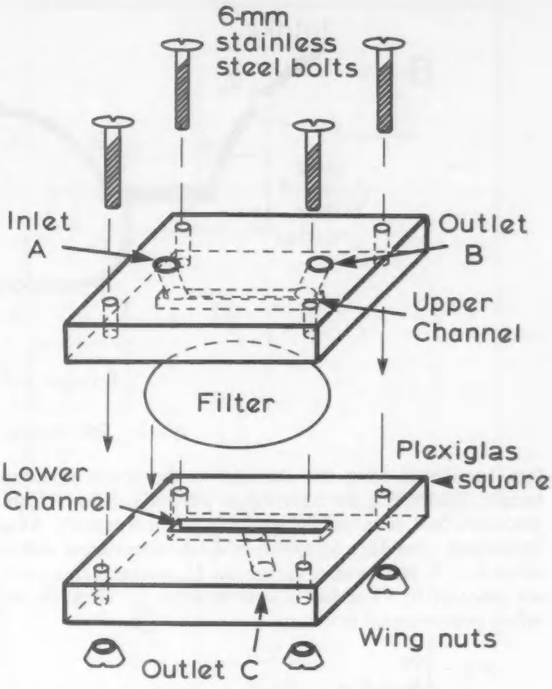

FIG. 1. Exploded view of Plexiglas filter holder.

Time Resolution

The time resolution of the continuous technique is limited by the response characteristics of the autoanalyzer itself. Due to mixing within the pump and reaction coils, a step change in the input sample concentration, S, does not produce a step change in the autoanalyzer signal, S_A. For ammonium analysis, the response can be approximated as a first-order linear one:

$$(1) \quad dS_A/dt = (S - S_A)/\tau.$$

For a step change in inflow concentration, the signal according to equation 1 approaches the new concentration exponentially, with characteristic time τ. For our system, τ is approximately 0.6 min.

If, in an uptake experiment, the intake is transferred directly from a baseline solution to the newly spiked sample, the first few minutes of uptake are lost as the signal responds to the initial step change in concentration. A better procedure is to run a standard made up to the same concentration as the proposed perturbation until the signal stabilizes at this level. The intake is

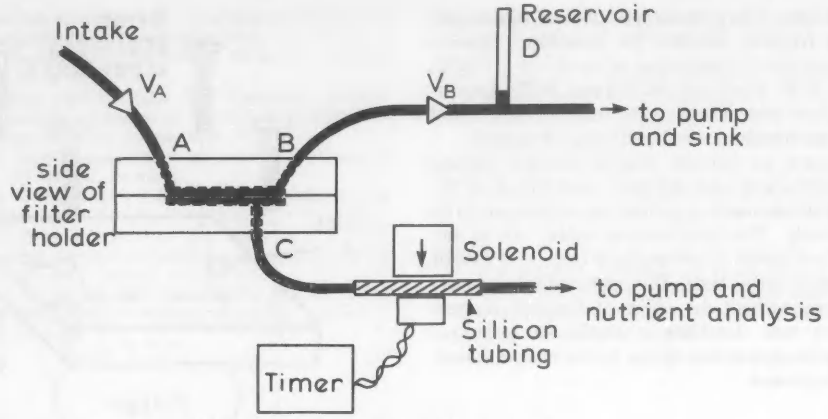

FIG. 2. Self-cleaning, in-line filter assembly.

then transferred from the standard to the newly perturbed sample, minimizing the error due to smearing. However, this procedure does not avoid the smearing problem entirely. As an illustration, consider a hypothetical uptake experiment with an initial level S_0 and constant uptake rate U, starting at time $t = 0$, and preceded by a standard of concentration S_0. Then, the true inflow concentration is

$$S(t) = \begin{cases} S_0 & t < 0 \\ S_0 - U \cdot t & t > 0. \end{cases}$$

The output signal, obtained by integrating equation 1, will be

$$S_A(t) = \begin{cases} S_0 & t < 0 \\ S_0 - Ut + U\tau(1 - e^{-t/\tau}) & t > 0. \end{cases}$$

These input and output concentrations are plotted in Fig. 3A. The apparent uptake rate U_A, calculated as the negative slope of the apparent nutrient concentration $S_A(t)$, is

$$\begin{aligned} U_A &= dS_A/dt \\ &= U(1 - e^{-t/\tau}). \end{aligned}$$

That is, U_A is initially zero and approaches the correct rate U with characteristic time τ.

The disappearance traces obtained in real uptake experiments with N-starved cultures of *T. pseudonana* (Fig. 3B) differ from the hypothetical one (Fig. 3A) in two respects. The slope U_A at $t = 0$ is not zero, but changes abruptly to a maximum value. This occurs because the culture concentration at $t = 0$ is already below S_0. Two factors contribute to this discrepancy. After the culture is perturbed, it must be mixed thoroughly before the intake is transferred. In addition, the time $t = 0$ on the output trace corresponds to the point at which the culture first reaches the filter. Uptake occurs both during mixing and while the culture travels along the intake tube to the filter, a combined lag of about 15 s.

The trace for *T. pseudonana* also differs from the hypothetical one because the real uptake rate is not constant with time. It is initially high and drops to a more or less constant value after approximately 10 min. To show that this is not simply an artifact of the 15-s delay, one need only back-extrapolate the linear portion of the disappearance trace to $t = -15$ s (Fig. 3B). The intercept there is well below S_0, whereas it would lie at or above S_0 if the uptake rate were constant. Whereas the existence of an initial surge uptake can be inferred, its exact magnitude and timing are disguised by the combined effects of the 15-s delay and the autoanalyzer response characteristics.

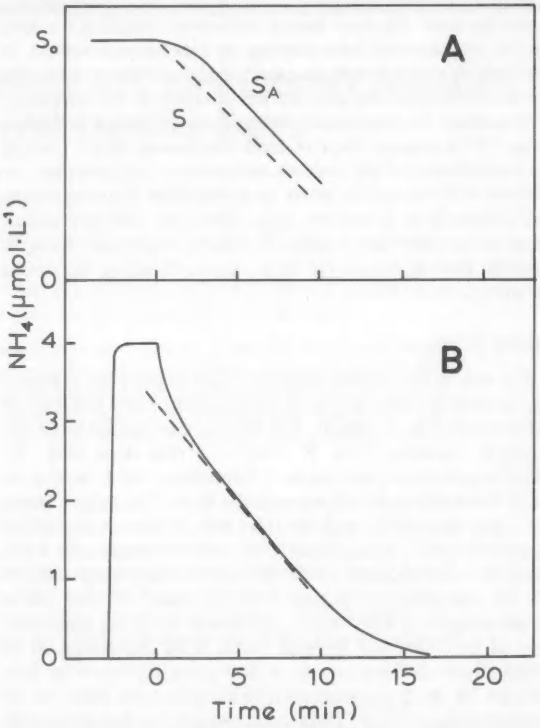

FIG. 3. Ambient ammonium concentration, S, and autoanalyzer response, S_A, for the hypothetical uptake experiment described in text. (B) Autoanalyzer response, S_A, for an ammonium uptake experiment using a 24-h starved, batch culture of *T. pseudonana* (50 µmol particulate N·L^{-1}).

In principle, one can correct for the autoanalyzer response characteristics. Equation 1 can be rewritten to give the corrected nutrient concentration

$$\hat{S} = S_A + \tau \cdot dS_A/dt$$

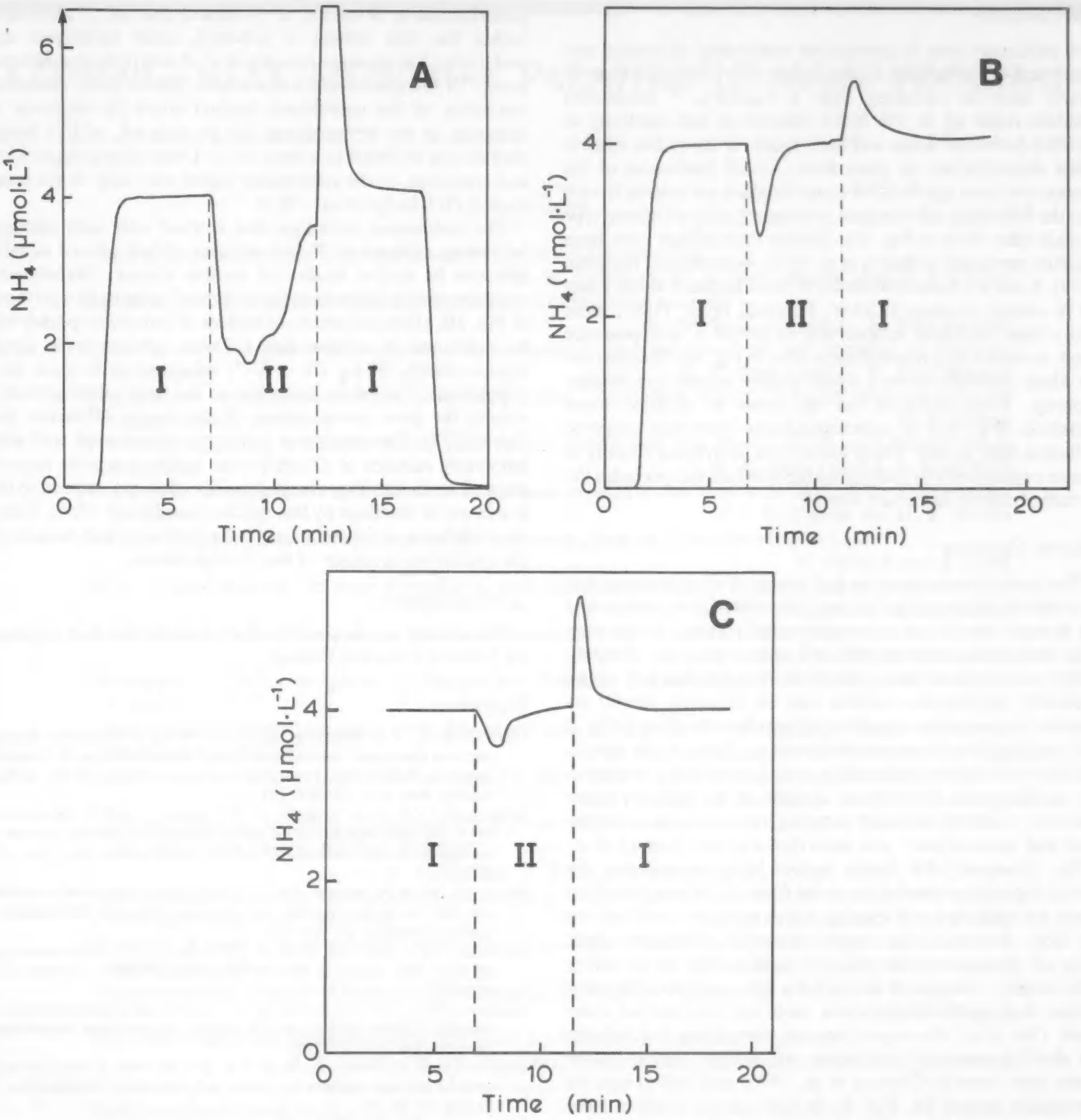

FIG. 4. (A) Autoanalyzer response, S_A, with a filter (Gelman GF/A) in line, when intake is transferred from a 4 μmol·L^{-1} ammonium standard made up in 3% NaCl solution (I) to one made up in distilled, deionized water (II) and back again. (B) As in Fig. 4A, but with a nitrocellulose filter and standard II made up in ammonium-depleted, artificial seawater medium. (C) As in Fig. 4A, but with a polycarbonate filter.

and the corrected uptake rate is then

$$\begin{aligned}\hat{U} &= -d\hat{S}/dt\\ &= -dS_A/dt - \tau \cdot d^2S_A/dt^2.\end{aligned}$$

In practice, this means digitizing the autoanalyzer signal (presently recorded on chart paper) and applying the correction numerically. Unfortunately, errors involved in calculating the second derivative of the digitized trace have been too high to provide a useful correction in the first minute or two, where the correction is important. One can calculate the likely systematic errors involved in using the uncorrected signal $S_A(t)$, given the time dependence of uptake, by integrating equation 1. Fortunately, the errors due to smearing and due to the 15-s lag tend to cancel. For values of τ and uptake rates appropriate to our experiments, the average uptake rate over 0–1 min is underestimated by about 20%. The rate from 1 to 2 min is underestimated by about 5% and that from 2 to 5 min by about 3%. Errors after 5 min are negligible. The continuous technique therefore currently offers rough estimates of uptake rates over the first minute and good estimates of uptake rates in subsequent intervals. It cannot resolve changes in uptake rate within the first minute.

Filter Sorption

A cautionary note is appropriate concerning adsorption and desorption of ammonium by the in-line filter. This effect can be clearly seen on switching from a 4 μmol$\cdot$L^{-1} ammonium standard made up in 3% NaCl solution to one made up in distilled deionized water and back again. If the in-line filter is either nitrocellulose or glass fiber, it will bind most of the ammonium from the distilled water standard and release it back into the following salt standard, as illustrated for a Gelman type A glass-fiber filter in Fig. 4A. Similar filter effects have been reported previously (Marvin et al. 1972; Schierup and Riemann 1979). Even in a transfer from a 3% NaCl standard to one made up in culture medium (ESAW, Harrison et al. 1980), these filters bind sufficient ammonium to distort a disappearance trace, as shown for a nitrocellulose filter in Fig. 4B. Polycarbonate filters generally show a much smaller ammonium binding capacity. Their effect in the salt water to distilled water transition (Fig. 4C) is indistinguishable from that observed without a filter in line. These effects can be avoided entirely in uptake experiments by making up the standard that precedes the perturbed culture in culture filtrate.

Discrete Sampling

The nutrient concentration and its rate of disappearance can be controlled by varying the size of the initial perturbation and the biomass density of the experimental culture. Where very long incubations are required, and uptake rates are changing slowly, a continuous trace of nutrient concentration may not be necessary. A discrete version can be obtained simply by inserting an automatic sampler upstream from the filter in Fig. 2 and sampling the culture at fixed intervals. This uses less sample and, by retaining the in-line filter, avoids error that may arise in the handling and filtration of discrete subsamples by other methods. A similar, discrete sampling system, using an in-line filter and autoanalyzer, was described earlier (Conway et al. 1976). However, this earlier system lacked any device for preventing cell accumulation on the filter. It did use a distilled water wash between subsamples, which probably lysed cells on the filter, preventing the continued uptake of nutrient. Still, there are reasons to prefer the self-cleaning filter to the earlier (and simpler) system. As cells on the filter are lysed, they will release their internal ammonium pools into the distilled water wash. One might also expect internal ammonium released into the distilled water to bind to the in-line filter (nitrocellulose filters were used by Conway et al. 1976) and desorb into the subsequent sample (cf. Fig. 4). In fact, sample peaks in these experiments did show trailing shoulders or trailing spikes and occasionally leading spikes later in the experiments (P. J. Harrison, unpubl. data). Estimates of uptake rates would be distorted to the extent that the "true" peak height could not be distinguished from the artifacts.

Discussion

The continuous technique allows uptake rates to be measured as disappearance of nutrient from the medium with relatively fine time resolution (approximately 1 min) for periods up to several hours. It is most useful for relatively short incubations (of 10- to 40-min duration), which in our experience cover the period of rapid changes in ammonium uptake rates following perturbations to N-starved or N-limited cultures. If resolution within the first minute is required, other techniques are preferable. Uptake rates over the first 15 s have been estimated using ^{15}N incorporation (Goldman and Glibert 1982). The time resolution of the continuous method could be improved if smearing in the autoanalyzer can be reduced, or if a better method can be found to correct for it. Direct digital acquisition and correction of the colorimeter signal may help in the latter respect (Whitledge et al. 1981).

The continuous technique has worked well with unialgal laboratory cultures of *T. pseudonana*. It has proved equally effective in similar studies of another diatom, *Skeletonema costatum*, which displays uptake kinetics comparable with those of Fig. 3B. (Detailed results of studies of both these species will be published at a later date.) These cultures were dense (approximately 50 μg chl $a\cdot$L^{-1}) compared with most field populations. Use of the technique in the field would probably require the prior concentration of the sample (Harrison and Davis 1977). The continuous technique did not work well with laboratory cultures of *Olisthodiscus luteus*, a marine chrysophyte. The in-line filter clogged rapidly, due, we suspect, to the exudation of mucilage by this species (Leadbeater 1969). Thus, the usefulness of the technique in the field may also depend on the species composition of the phytoplankton.

Acknowledgments

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R. W. Newbury and G. K. McCullough

Effect of impoundment and diversion on the sediment budget and nearshore sedimentation of Southern Indian Lake
R. E. Hecky and G. K. McCullough

Thermal and optical characteristics of Southern Indian Lake before, during, and after impoundment and Churchill River diversion
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Primary productivity of Southern Indian Lake before, during, and after impoundment and Churchill River diversion
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